

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Lorraine Faxon Meisner
Application No: 12/015,258
Filed: January 16, 2008
For: COMPOSITIONS AND METHODS FOR THE TREATMENT
OF SKIN
Examiner: Choi, Frank I.
Art Unit: 1616

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Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Declaration of Dr. Lorraine Faxon Meisner under 37 C.F.R. § 1.132

I, Dr. Lorraine Faxon Meisner, residing at 137 East Wilson Street, Unit 510, Madison, Wisconsin, hereby declare as follows:

I. Background

1. I earned a M.A. from the Department of Anthropology at the University of Chicago in 1958, and then went on to earn a Ph.D. in Genetics and Cell Biology from the University of Chicago in 1966.

2. I am currently a Professor Emerita at the University of Wisconsin-Madison, and have held this position since 2006. I taught courses at the University of Wisconsin-Madison Medical School from 1968 to 2001. I started out as an Instructor of Preventive Medicine in 1968, and was subsequently promoted to Assistant Professor in

1970, Associate Professor in 1974, and full Professor in 1991. I have also held the positions of Professor of Population Health Science, Professor of Pathology and Laboratory Medicine, and Associate Professor of Human Oncology at the University of Wisconsin-Madison Medical School.

3. I am a member of the American Society of Cell Biology, the American Association for the Advancement of Science, the American Society of Human Genetics, the New York Academy of Sciences, and Sigma Xi. I am also a Fellow of the American Board of Medical Genetics.

4. I have reviewed U.S. application Serial No. 12/015,258 ("the '258 application," attached hereto as Exhibit A), entitled "Compositions and Methods for the Treatment of Skin" and assigned to Bioderm, Inc. I am the President of Bioderm, Inc. and am named as the sole inventor of the subject matter claimed in the '258 application.

II. Murad Does Not Teach How to Prepare a Topical Composition of Ascorbic Acid and Glucosamine

5. I have reviewed U.S. Patent No. 5,804,594 to Murad ("Murad," a copy of which is attached hereto as Exhibit B).

6. Murad does not disclose how to prepare a composition of ascorbic acid and glucosamine for topical administration.

7. Moreover, Murad does not provide any guidance on how to adapt its compositions for topical administration.

8. Murad's examples are limited to oral tablets and capsules, which are unsuitable for topical administration. Topical compositions require different formulation considerations than do oral compositions. For example, a composition consisting essentially of: at least 10% (w/v) ascorbic acid; approximately 10% to 25% (w/v) glucosamine; and water, wherein the composition has a pH of about 3.5 to about 4.1, when topically applied, is capable of treating rosacea or acne. On the other hand, the oral tablets and capsules exemplified by Murad could not produce the same effect when topically applied. Thus, one cannot formulate a topical composition by simple

extrapolation from an oral composition having the same active ingredient. This is shown, for example, by the following experiments that I have performed.

9. I mixed together therapeutically effective amounts of ascorbic acid and glucosamine powders in an attempt to make a paste for topical administration. Admixing 10g of ascorbic acid (14% of the total composition), 10g of glucosamine (also 14% of the total composition), and 50g of a basic commercial cream base (more than half of which was water) initially yielded a tan colored cream. However, upon standing overnight in a closed reaction vessel, a vigorous, exothermic reaction occurred during which the composition expanded, blew the lid off the reaction vessel, and turned dark brown. I repeated this experiment by admixing 5g of ascorbic acid (10% of the total composition), 5g of glucosamine (also 10% of the total composition), and 40g of cream base, and the same result occurred. I further repeated the experiment using an open reaction vessel to allow the gas evolved during the exothermic reaction to escape. However, upon standing overnight in the open reaction vessel, the composition still expanded and turned dark brown.

10. The above experiments illustrate that, to the extent, if any, Murad suggests combining ascorbic acid and glucosamine, the combination results in an unstable mixture that is unsuitable for topical administration.

III. Prior to my Invention, One Would Have Expected an Ascorbic Acid Composition to be Unstable at a pH of about 3.5 to about 4.1

11. It was well-known that ascorbic acid is unstable at pH between about 3 and 4. This is evidenced by several publications, including the following:

- J.C. Bauernfeind, "Ascorbic acid technology in agricultural, pharmaceutical food, and industrial applications," in ASCORBIC ACID: CHEMISTRY, METABOLISM, AND USES, 417-429 (P.A. Seib & B. M. Tolbert, eds., 1982) (a copy of which is attached hereto as Exhibit C) states:

Ascorbic acid degradation is also pH dependent. Under aerobic conditions, the rate of oxidation shows maxima at pH 5 . . . and at pH 11.5 . . . Under anaerobic conditions, the dependency of the stability of ascorbic acid in aqueous

solutions on pH is relatively low, but ***there is a maximum rate of degradation, which is equal to the pK_{a1} of ascorbic acid, at a pH of about 4.1.*** Stability of ascorbic acid in multivitamin drops has been studied at various pH levels. ***Maximum losses occur in the pH range of 3.5 to 4.5*** and smaller losses are found at higher pH (up to 5.5).” (p. 420, emphases added).

- B.R. Hajratwala, “Stability of ascorbic acid,” *S.T.P. Pharma*, 1(4): 281-286 (1985) (a copy of which is attached hereto as Exhibit D) states:

Figure 1 shows the pH-log rate profile for the ascorbic acid decomposition in aqueous solutions . . . Ascorbic acid decomposition shows minima around pH 2.5 to 3, followed by ***a maxima around pH 4.0*** and another minima around pH 6.0 to 6.5.” (pp. 282-283, emphasis added).

Figure 1 is reproduced below.

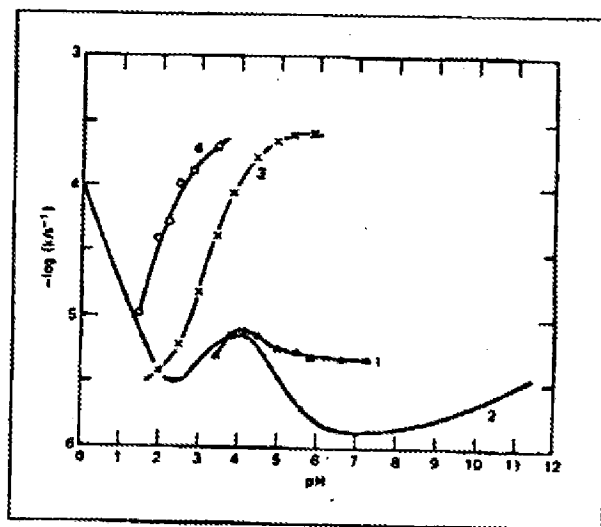
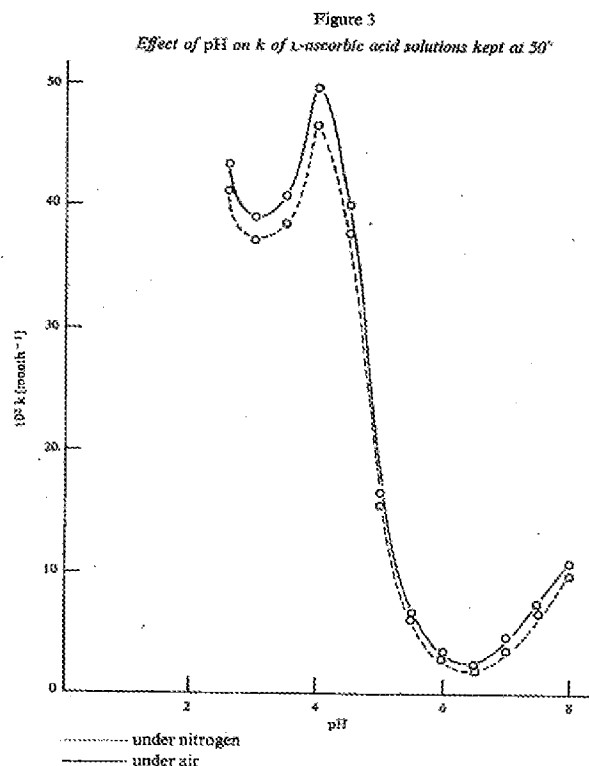


Figure 1 — pH-log rate profiles for ascorbic acid decomposition in aqueous solutions. Curve 1, aerobic, 67°C, $\mu = 0.4$ (Reference [6]); Curve 2, anaerobic, 95°C, $\mu = 0.5$ (Reference [7]); Curve 3, saturated with O_2 , 25°C, $\mu = 0.1$ (Reference [17]); Curve 4, saturated with O_2 and in the presence of $5 \times 10^{-5} M Cu^{2+}$, 25°C, $\mu = 0.1$ (Reference [17]). Reproduced from reference [5] with the permission of the copyright owner (John Wiley & Sons).

- M.A. Kassem, et al., “Studies on the stability of injectable L-ascorbic acid solutions. I. Effect of pH, solvent, light and container,” *Pharm. Acta Helvetica*, 44: 611-628 (1969) (a copy of which is attached hereto as Exhibit E) states:

On autoclaving, it is obvious that a pH-dependent loss of L-ascorbic acid takes place (table 1). ***The highest loss in potency takes place at pH = 4 . . .*** The reaction rate constant (k) is found to be highly pH-dependent (table 2 and fig. 3). ***The highest values of k are observed in the pH range 2,6 to 4,5.*** However, a minimum occurs at pH = 3, which thus represents a small maximum of stability in the above mentioned range. The reaction rate attains its highest value at pH = 4. (p. 613, emphases added).

Figure 3 is reproduced below.



12. In view of the above-described publications, prior to my invention one would have expected an ascorbic acid composition to be unstable at a pH of about 3.5 to about 4.1.

Appl. No. 12/015,258
Filed January 16, 2008
Rule 132 Declaration of Dr. Lorraine Faxon Meisner

I declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. §1001 and that such willful false statements may jeopardize the validity of any United States Patent that would issue from U.S. application Serial No. 12/015,258.

Dated: April 9, 2009

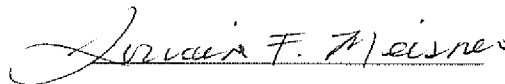

Dr. Lorraine Faxon Meisner

EXHIBIT A



US 20080125395A1

(19) **United States**(12) **Patent Application Publication**
Meisner(10) **Pub. No.: US 2008/0125395 A1**(43) **Pub. Date: May 29, 2008**(54) **COMPOSITIONS AND METHODS FOR THE
TREATMENT OF SKIN**(76) Inventor: **Lorraine Faxon Meisner,**
Madison, WI (US)

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WILSON SONSINI GOODRICH & ROSATI
650 PAGE MILL ROAD
PALO ALTO, CA 94304-1050(21) Appl. No.: **12/015,258**(22) Filed: **Jan. 16, 2008****Related U.S. Application Data**(63) Continuation of application No. 09/997,663, filed on
Nov. 29, 2001, which is a continuation-in-part of appli-
cation No. 09/732,385, filed on Dec. 7, 2000, now Pat.
No. 6,444,699, which is a continuation of application
No. 09/356,142, filed on Jul. 19, 1999, now Pat. No.
6,217,914.(60) Provisional application No. 60/125,356, filed on Mar.
19, 1999.**Publication Classification**(51) **Int. Cl.****A61K 31/70** (2006.01)**A61P 17/00** (2006.01)(52) **U.S. Cl. 514/62**

(57)

ABSTRACT

An ascorbic acid-based composition and related method for the treatment of aging, photo-damaged or inflamed skin is disclosed. The composition includes water and ascorbic acid, at least a portion of which has generally been pretreated by being dissolved under relatively high temperature and concentration conditions. The composition typically includes at least about 5.0% (w/v) ascorbic acid formulated to have a pH above 3.5. 10 to 50% of the ascorbic acid is pretreated ascorbic acid. The composition may also include a non-toxic zinc salt, a tyrosine compound, and/or pharmaceutically acceptable carrier. The composition may include an anti-inflammatory compound, such as aminosugar and/or sulfur-containing anti-inflammatory compound. Embodiments containing an aminosugar such as glucosamine are further useful for treating rosacea and other inflammatory skin ailments. The composition may be administered in a variety of forms suitable for topical application on skin.

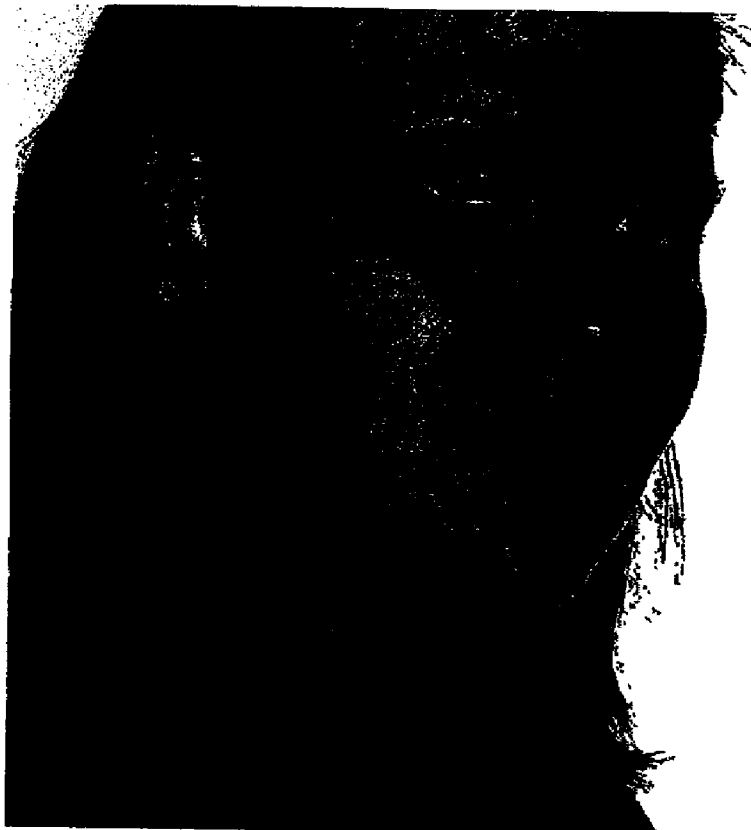


FIG. 1

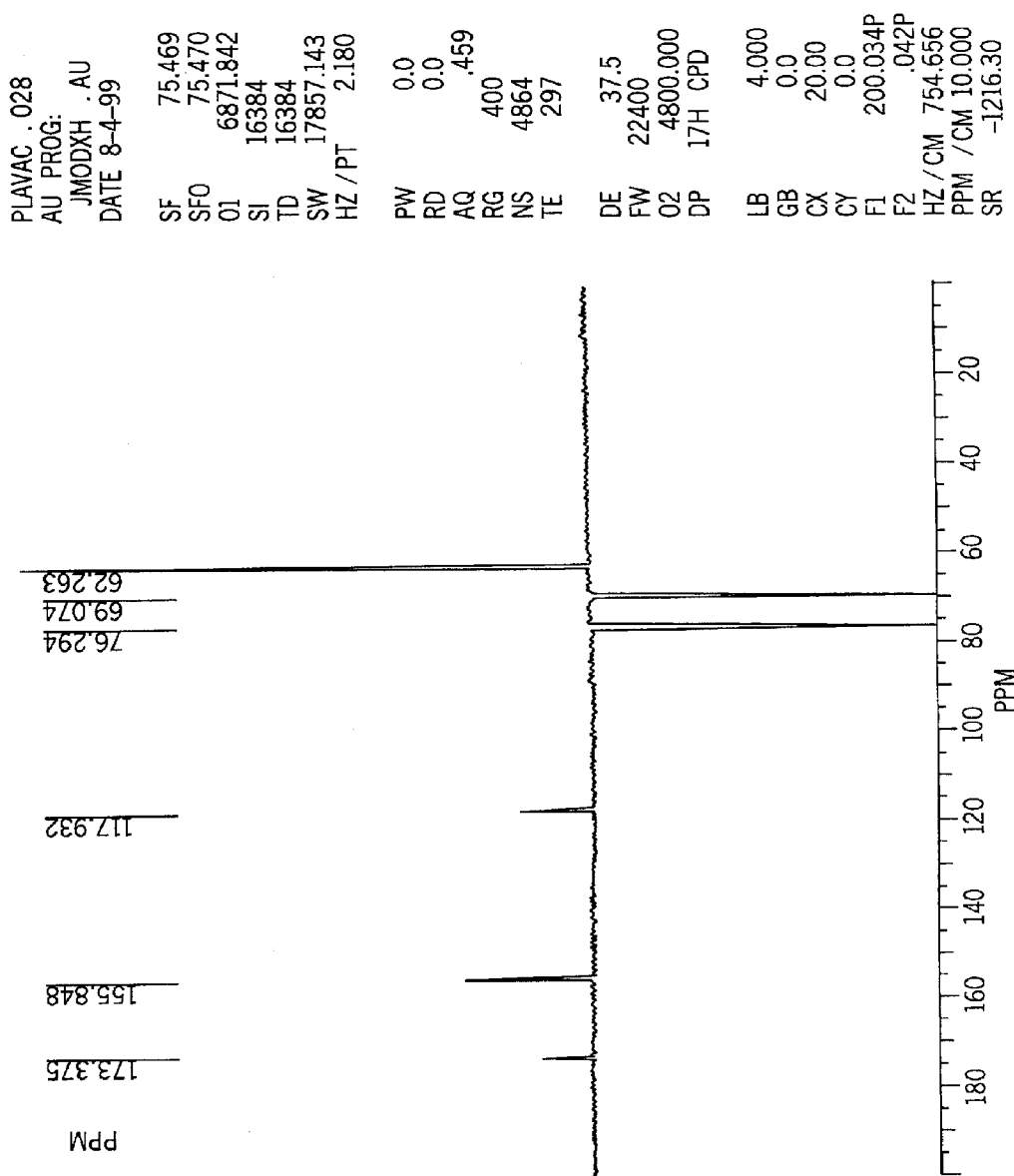


FIG. 2

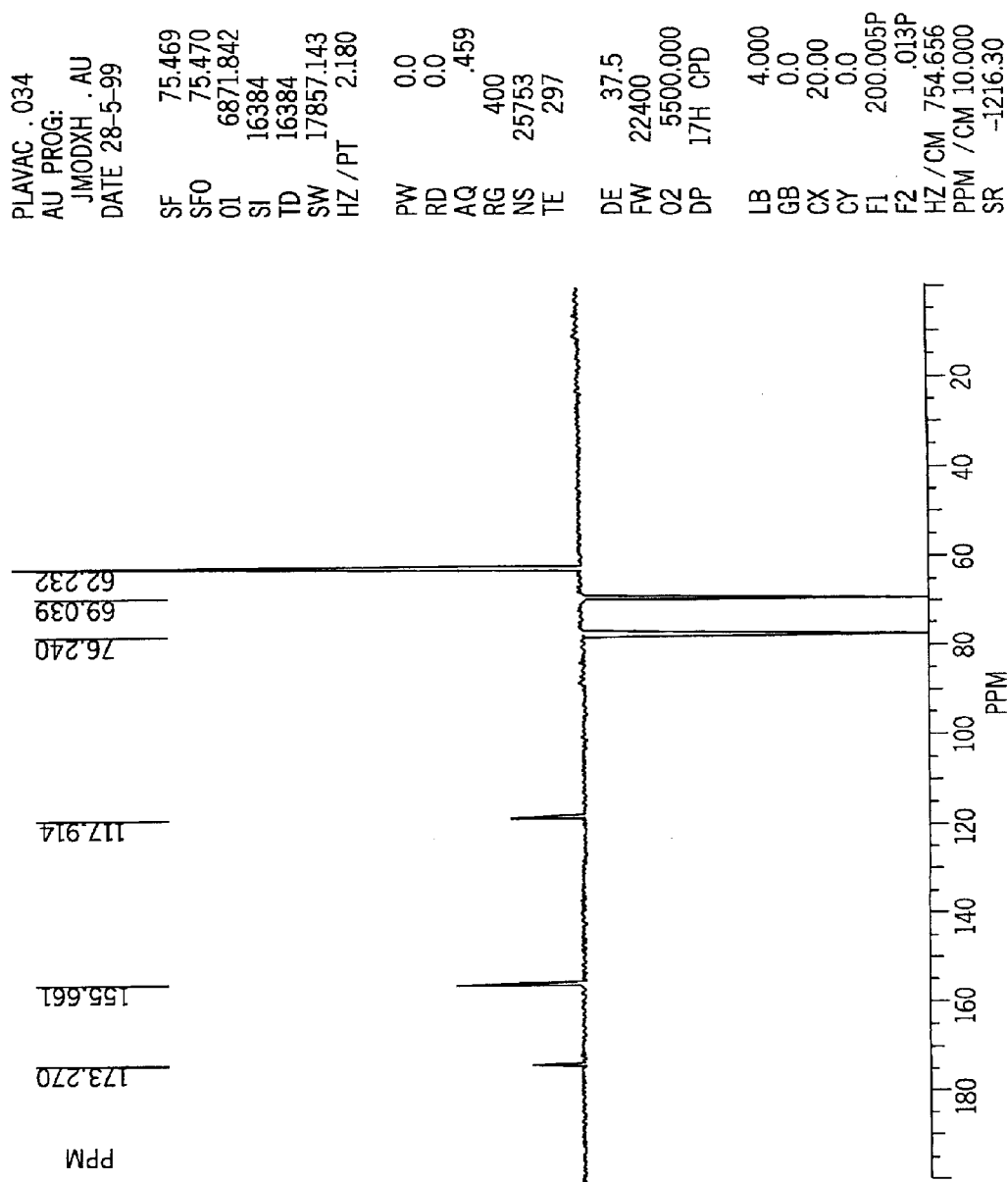
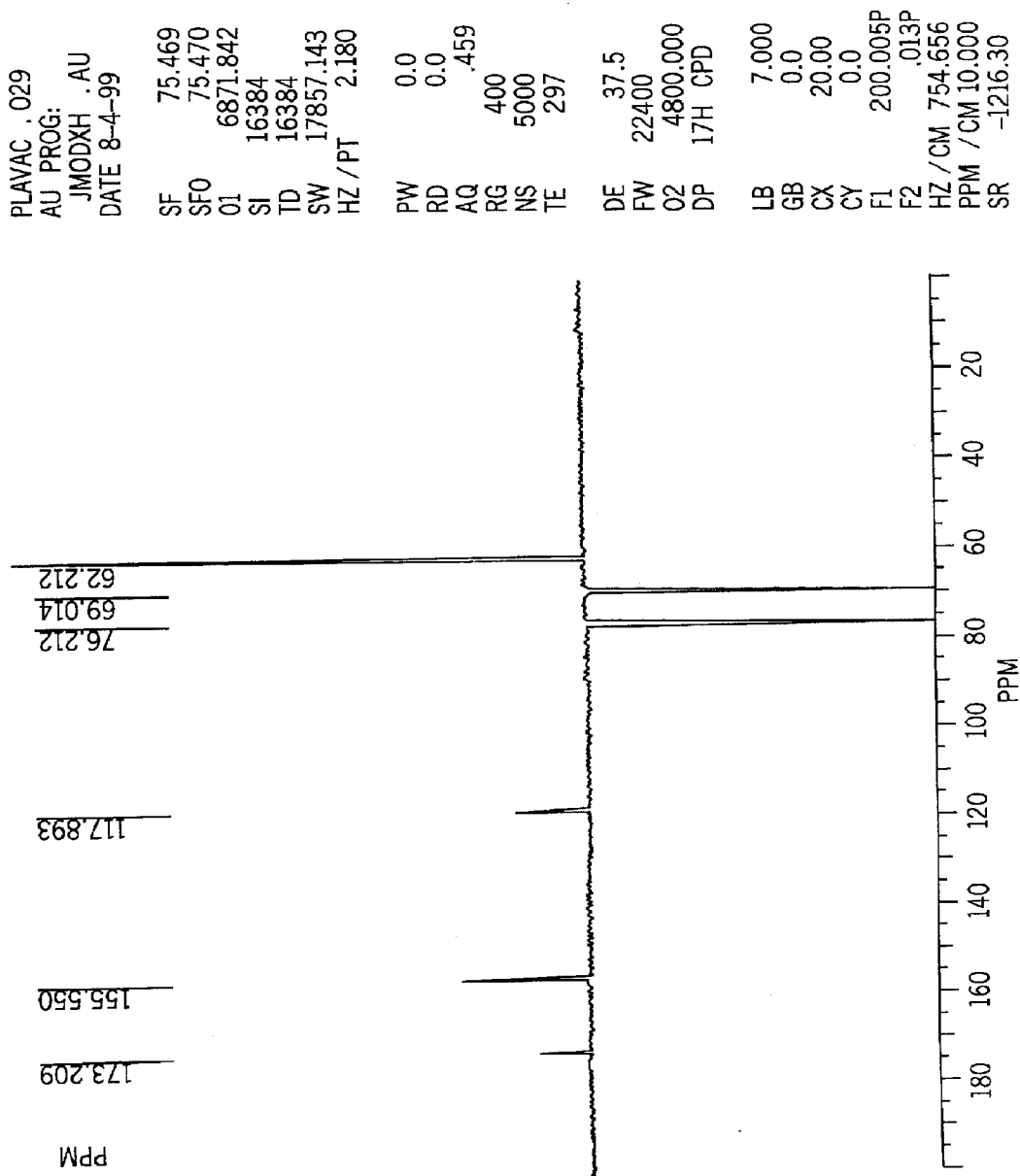
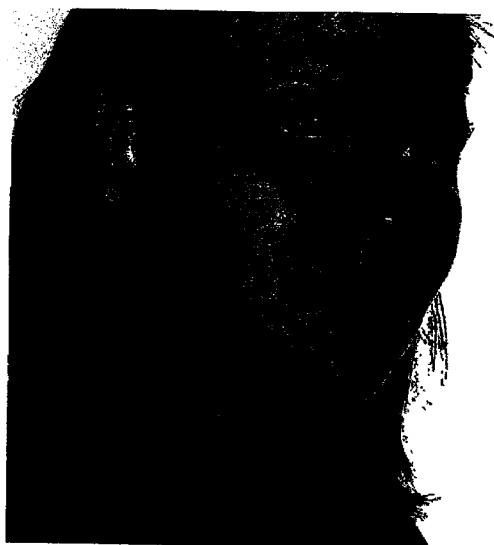
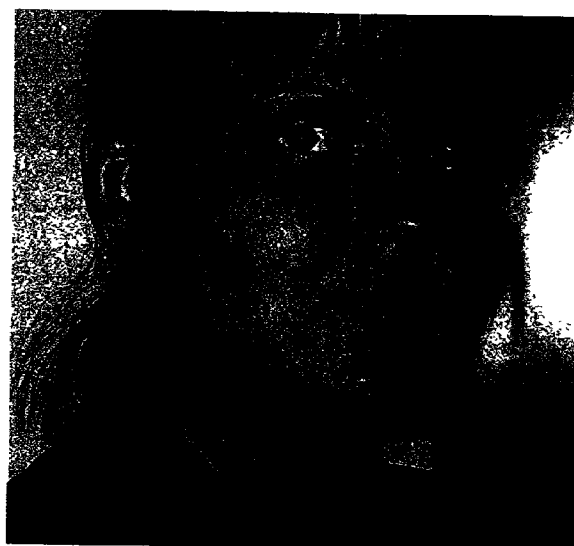


FIG. 3





A



B

Fig. 4

COMPOSITIONS AND METHODS FOR THE TREATMENT OF SKIN

[0001] This application is a continuation of application Ser. No. 09/997,663, filed on Nov. 29, 2001, which is a continuation-in-part of application Ser. No. 09/732,385, filed on Dec. 7, 2000, now U.S. Pat. No. 6,444,699, which is a continuation of application Ser. No. 09/356,142, filed Jul. 19, 1999, now U.S. Pat. No. 6,217,914, which claims the benefit of Provisional Application Ser. No. 60/125,356, filed on Mar. 19, 1999, the disclosure of each of which is incorporated by reference herein in its entirety.

BACKGROUND OF THE ART

[0002] Skin is composed of a top layer, the epidermis, which is approximately 20 cell layers or about 0.1 mm in thickness, and a lower layer, the dermis, which is from about 1 to about 4 mm in thickness and contains small blood vessels, collagen, elastin and fibroblasts. The dermis provides structural support and nutrients to the epidermis. Aging has been shown to increase cellular heterogeneity of the epidermal layer, however, it has little effect on the thickness of the epidermal layer. The supporting dermis, on the other hand, is known to thin with age and exposure to the sun and environmental contaminants. The dermal layer provides the support and blood supply for the epidermis, therefore the dermal layer is important in maintaining the elasticity and appearance of the skin. Disruption of the supporting dermis leads directly to sagging and consequent furrowing of the epidermis, i.e., the formation of wrinkles.

[0003] Deep wrinkles are also due to continual stretching and contraction of both the dermis and epidermis. Currently, these deep wrinkles or furrows may only be eliminated by plastic surgery or by collagen injections directly beneath the depressed areas. The fine wrinkles that occur with age and prolonged exposure to the sun and other environmental contaminants are the direct result of deterioration of the supporting dermal layer. Other environmental effects on the skin are discussed in U.S. Pat. Nos. 4,938,969 and 5,140,043.

[0004] As a result of the aging process and damage caused by incident radiation, a disruption of the collagen bundles that provide support to the epidermis is observed. Collagen exists normally in dense, organized patterns. During the aging process collagen becomes disorganized and less supportive of the epidermis and the dermis loses elasticity. There is also progressive loss of circulatory support from the small blood vessels that are more numerous and close to the surface in young skin. The result of aging on skin, whether or not it has been accelerated by incident radiation, is a deterioration of the dermal layer—fewer fibroblasts, less collagen, less elastin and less circulatory support. Consequently, the normal stretching and contraction of the skin leads to damage of the dermis that is not readily corrected and wrinkling results.

[0005] Dermatologists and cosmetologists have directed their efforts to improving the appearance of skin using agents known to stimulate the growth and proliferation of epidermal cells. Newly proliferated cells provide more structure and hold more moisture, giving the skin a younger appearance. One method of causing new skin cell proliferation is accomplished by use of an irritant or chemical peel in which the uppermost layers of the epidermis are caused to slough off, leading to proliferation and replacement with new epidermal cells. While such treatment is recognized to provide some

cosmetic improvement, it does not address the major causative factor, namely, the compromised supporting dermal layer.

[0006] Considerable effort has also been expended to find ways to prevent adverse changes in the skin brought about by ultraviolet (UV) exposure. Preventative approaches include physically blocking or absorbing the UV radiation before it can enter the skin using UV absorbing compounds. Blocking and absorbing are effective but are cumbersome because sun blockers or absorbers must be applied before every exposure and may be washed off with water. For example, after swimming UV absorbing compounds must typically be reapplied. Further, the long-term side effects of many of the compositions containing sun blockers and/or absorbers are not known.

[0007] L-ascorbic acid has many known biological functions from enzymatic cofactor to “sparing” agent against vitamin E depletion. See, for example, Englund and Seifter, “The Biochemical Functions of Ascorbic Acid,” *Ann. Rev. Nutri.* 6:365-406, (1986); Kunert and Tappel, “The Effect of Vitamin C on in vivo Lipid Peroxidation in Guinea Pigs as Measured by Pentane and Ethane Production,” *Lipids* 18:271-74 (1983). The latter function of L-ascorbic acid may partly account for its “anti-oxidant” status. Additionally, at higher concentrations, ascorbic acid is known to react with both the super oxide and hydroxyl radicals. The super oxide, and the hydrogen peroxide and hydroxyl radical subsequently generated, are oxygen-containing free radicals now known to be generated in vivo under a variety of normal and pathological conditions. These radicals have been implicated as causative agents for everything from sunburn to aging and are believed to destroy lipid membranes, break down DNA and inactivate enzymes, among other effects. An immense amount of work has been done in the last two decades documenting the deleterious behavior of oxygen radicals. Several recent texts on the subject include: *Oxy-radicals in Molecular Biology & Pathology*, D Cerutti, I. Fridovich, J. McCord, eds., (Alan R. Liss, Inc. New York, 1988); *Biological Role of Reactive Oxygen Species in Skin*, O. Hayaishi, S. Inamura, Y. Mayachi, eds. (Elsevier Press, New York, 1987); *Free Radicals, Aging and Degenerative Diseases*, J. E. Johnson, Jr., R. Walford, D. Harmon, J. Miguel, eds. (Alan Liss, Inc., New York, 1986); *Free Radicals in Biology and Medicine*, B. Halliwell and J. M. C. Gutteridge, eds., (Clarendon Press, Oxford, 1985); and *Oxidative Stress* Helmut Sies, ed. (Academic Press, 1985). Also addressing the subject are several symposia, including “Oxygen Radicals and Tissue Injury” Proceedings from an Upjohn Symposium (April, 1987); and “Oxygen Free Radicals,” Proceedings from National Heart, Lung & Blood Institute (National Institute of Health, Washington, D.C., December 1987).

[0008] As a result of the known effects of the use of ascorbic acid on damaged and aging skin, there are now various Vitamin C or ascorbic acid ointments, serums and creams that are used with varying degrees of success to prevent and/or repair damage to the skin’s dermal layer. For example, it has been reported that a composition including ascorbic acid, tyrosine and a non-toxic zinc salt, preferably zinc sulfate, in a vehicle suitable for topical application, when applied to areas showing the fine wrinkles associated with aging/sun exposure, results in a readily perceivable diminution of the fine wrinkle structure. It has also been reported that ascorbic acid topical aqueous compositions are unstable unless maintained at a pH below about 3.5. Topical compositions containing a

carrier and a concentration of L-ascorbic acid above about 1% (w/v) may be stable if maintained at a pH below about 3.5 or even below about 2.5.

[0009] In addition to ameliorating skin damaged by age or environmental insult, topical ascorbic acid formulations may also exhibit some effectiveness in the treatment of various inflammatory skin maladies such as, for example, inflammatory rosacea, allergic inflammations and hypersensitivity. A few cases in which a topical formulation of ascorbic acid, zinc and tyrosine was applied to rosacea, for example, a slight improvement was observed with long-term use. Such formulations, however, have not been shown to clear up rosacea to a substantial extent and even slight improvement requires long-term, diligent daily application to the affected skin. Improved formulations of topical ascorbic acid are needed to effectively and quickly treat skin maladies such as rosacea, allergic inflammations and hypersensitivity.

[0010] Currently available ascorbic acid compositions and methods fail to provide the delivery system for formulations having the desired combination of efficacy, non-irritability, stability and convenient storage solutions for topical Vitamin C applications. A significant problem of current compositions is that it is not practical to use more than 15% (w/v) ascorbic acid in a serum, cream gel or other suitable topical formulation for cosmetic use because the low inherent pH (circa 2-2.5) of such a formulation is often quite irritating to the skin. The breakdown of the ascorbic acid in such low pH formulations due to exposure to water, heat, and air can also lead to undesirable discoloration and eventually loss of efficacy. Furthermore, if the ascorbic acid is formulated in a cream with limited water content to enhance stability of the ascorbic acid over time, changes in heat, atmospheric pressure and/or moisture content may activate the ascorbic acid, leading to unacceptable expansion and even explosion of the containers holding such creams or gels. There is, therefore, a continuing need for topical ascorbic acid-based compositions that improve the efficacy and stability of such skin treatment formulations.

SUMMARY OF THE INVENTION

[0011] The present invention provides stable, effective topical compositions that include ascorbic acid, generally in a relatively high pH formulation. The concentration of active ascorbic acid that is available to be delivered to the skin is maintained at a high concentration, while at the same time lowering the irritating effects commonly associated with aqueous compositions having a high concentration of organic acid. By providing, for example, a portion of the total ascorbic acid of the composition in the ascorbate salt form, the composition disclosed herein decreases the overall irritant nature of the solution without losing efficacy or desired biological effect. The present ascorbic acid-based compositions are particularly effective for topical application to reduce epidermal wrinkling, such as that resulting from intrinsic aging or photo damage. For example, applying the present compositions within about six hours to skin that has received excess sun damage can attenuate the effects due to UV exposure and decrease sunburn and cell damage. In addition, the compositions disclosed herein did not expand or lose integrity on storage. The present compositions were also far less likely to oxidize to yield an off color (e.g., to become darker or brown). Subjects using the present ascorbic acid formulations found the product to be very effective, and to yield rapid results relative to decreasing the appearance of fine lines.

[0012] In addition to treating aged or damaged skin, the present invention further includes compositions and methods for treating inflammatory skin ailments such as inflammatory rosacea. The present invention provides formulations of ascorbic acid in combination with an anti-inflammatory agent such as, for example, glucosamine or other suitable aminosugar. Such combination formulations demonstrate significant improvement in the treatment of inflammatory ailments of the skin such as, for example, rosacea, in comparison to formulations of ascorbic acid without, for example, glucosamine. Formulations of the present invention that include glucosamine are particularly useful over prior art formulations for treating inflammatory ailments of the skin that have demonstrated resistance to prior art treatments.

[0013] Conventional treatment for rosacea is cortisone therapy. Long-term use of cortisone is undesirable, however, because it weakens the strength and resilience of connective tissue. Another drawback of cortisone use is the rebound effect that occurs when the use of cortisone is temporarily discontinued. The present invention provides a treatment for rosacea that does not have the undesirable effects of cortisone and that is effective for individuals that are resistant to cortisone treatment. Methods are provided to combine glucosamine, for example, with ascorbic acid so that the ingredients maintain efficacy and achieve a stable shelf life in formulations suitable for topical application to the skin.

[0014] The present compositions typically include up to about 50% of the total ascorbic acid present that has been prepared by dissolution in water at relatively high temperature and concentration. Ascorbic acid dissolved in this manner is referred to herein as "pretreated" ascorbic acid and is prepared by dissolving a high concentration of ascorbic acid, typically at least about 20% (w/v) (i.e., at least about 200 mg/ml) in water at 60° to 90° C.

[0015] Importantly, compositions of a cream, or other suitable administration form of the present invention, containing 50% (w/v) ascorbic acid content in the form of pretreated ascorbic acid, formulated as described herein, are stable. That is, such compositions do not expand, explode, or discolor due to heat, changes in atmospheric pressure, or improper storage, all of which have proved to be problems in manufacturing, storing and distributing formulations of pure L-ascorbic acid and its direct break down products. Further, compositions of the present invention that comprise a combination of pretreated ascorbic acid and glucosamine are also stable.

[0016] Embodiments of the present compositions commonly include water, at least about 5.0% (w/v) ascorbic acid, and have a pH of more than 3.5. The compositions typically also include (a) non-toxic zinc salt and/or (b) a stimulant of protein synthesis and/or precursor to melanin synthesis (e.g., a tyrosine compound). The compositions may also include an anti-inflammatory compound, such as an aminosugar and/or a sulfur-containing anti-inflammatory compound. The topical compositions may be in any of a number of common forms, such as an aqueous solution ("a serum"), a hydrophilic lotion-, an ointment-, a cream or a gel. Typically, the topical composition includes a pharmaceutically acceptable carrier and may also include one or more other formulation additives, such as surfactant(s), thickener(s), dyes, other antioxidants and/or fragrance.

[0017] The "high pH" formulations of the present compositions are less irritating than high concentrations of L-ascorbic acid (with its inherent low pH, e.g., circa 2.0-2.5) because the relatively higher pH avoids the skin irritation problem

often encountered with harsh chemical peels or solutions with pH values below 3.5. The present compositions were also found to be very stable on short and long term storage, while maintaining a high degree of effectiveness.

[0018] The present invention also includes a method of treating damage to skin, such as often arises due ultraviolet light exposure and/or aging, as well as treating rosacea or other inflammatory skin afflictions. The method includes applying the present topical composition to a damaged or afflicted portion of the skin. For example, the present composition is typically applied topically to the locus of wrinkles or the locus of rosacea.

BRIEF DESCRIPTION OF THE FIGURES

[0019] The invention will become more fully understood from the following detailed description, taken in conjunction with the accompanying drawings, in which:

[0020] FIG. 1 shows a C^{13} NMR of a 10% (w/v) solution of "native" ascorbic acid after storage for one week at 37° C.

[0021] FIG. 2 shows a C^{13} NMR of a 1:1 mixture of a 10% (w/v) solution of "native" ascorbic acid and a 30% (w/v) solution of pretreated ascorbic acid after storage of the mixture (at pH 2.3) for one month at room temperature.

[0022] FIG. 3 shows a C^{13} NMR of a 30% (w/v) solution of pretreated ascorbic acid after storage for one week at 37° C.

[0023] FIG. 4 is a graphic rendering of before (A) and after (B) photographs of a subject who used an embodiment of the present invention.

DETAILED DESCRIPTION OF THE INVENTION

[0024] While the making and using of various embodiments of the present invention are discussed in detail below, it should be appreciated that the present invention provides many applicable inventive concepts that may be embodied in a wide variety of specific contexts. The specific embodiments discussed herein are merely illustrative of specific ways to make and use the invention and do not delimit the scope of the invention.

[0025] Long shelf-life and extended stability (e.g., for at least two years) is normally required for any cosmetic product to be distributed through ordinary channels in which there must be stored inventory to meet market demand without the concern that the inventory will deteriorate before being sold. The present ascorbic acid-based compositions have good efficacy and storage stability, and low skin irritability. These topical ascorbic acid based compositions are particularly effective for reducing epidermal wrinkling resulting from intrinsic aging or photo damage. The compositions of the present invention may also be used prophylactically to ameliorate the photo-induced damage that results from exposure of skin to sunlight and other harmful irradiation. Additionally, embodiments of the present invention in which ascorbic acid and glucosamine are combined are useful for treating skin afflicted with rosacea or other inflammatory skin conditions.

[0026] The compositions typically include at least about 5.0% (w/v) ascorbic acid. Herein, the amount of ascorbic acid present in a composition refers to the total amount of ascorbic acid and ascorbate present stated as if all was present in the acid form. In other words, a solution which includes 0.5 mole ascorbic acid and 0.5 mole of an ascorbate salt contains the same total amount of ascorbic acid as a solutions which include either 1.0 mole ascorbic acid or 1.0 mole of an ascorbate salt.

[0027] The present compositions commonly include at least about 5.0% (w/v) total ascorbic acid, which may comprise ascorbic acid in the form of monohydroascorbic acid or ascorbate salts. It is generally advantageous to include higher concentrations of ascorbic acid, typically at least about 10% (w/v) and often concentrations in the range of about 15 to about 25% (w/v) ascorbic acid. Because of the potential problems of skin irritation with formulations containing high concentrations of ascorbic acid, it is generally advantageous to adjust the pH of such formulations to at least about 3.5. To achieve an optimum combination of low irritability and high stability, the present compositions are typically formulated to have a pH of about 3.7 to about 4.1 and, for example, between about 3.8 to about 4.0. Compositions of the present invention may further include anti-inflammatory agents such as glucosamine in combination with ascorbic acid.

[0028] It has been found that ascorbic acid-based topical formulations in which a substantial portion of the ascorbic acid has been pretreated in accordance with the present invention exhibit particularly good storage stability. As noted above, for the purposes of this application, "pretreated" ascorbic acid also refers to ascorbic acid that has been dissolved in water at a relatively high temperature to form a concentrated ascorbic acid solution. Typically, the ascorbic acid is dissolved in water at between about 60 to about 90° C. (e.g., between about 75 to about 80° C.) to form a concentrated solution that contains at least about 20% (w/v) ascorbic acid. The ascorbic acid is dissolved in the acid form, i.e., the resulting solution will have a relatively low pH (circa 2.0-2.5). After dissolution, the concentrate is generally heated for an additional period of time (e.g., 0.25 to 1.0 hour) and cooled to below about 40° C. before being incorporated into the final formulation. If the pretreated ascorbic acid concentrate is to be stored prior to formulation, it may be stored at room temperature or below (e.g., about 3 to about 20° C.) and/or under conditions that exclude oxygen-containing gases such as air (e.g., in a sealed container or blanketed with an inert gas such as argon or nitrogen). In the present compositions, at least about 10% of the ascorbic acid present may be pretreated ascorbic acid. Typically, no more than about 50% of the ascorbic acid present has been pretreated to obtain the enhanced stability properties of the compositions while minimizing the additional processing steps and costs associated with the pretreatment of the ascorbic acid.

[0029] To test and quantitate the stability of composition containing pretreated ascorbic acid, nuclear magnetic resonance (NMR) spectra of stored samples of the following ascorbic acid-based solutions: (i) a 10% (w/v) solution of "native" ascorbic acid; (ii) a 1:1 mixture of the 10% (w/v) solution of "native" ascorbic acid and a 30% (w/v) solution of pretreated ascorbic acid; and (iii) the 30% (w/v) solution of pretreated ascorbic acid after storage. The results, shown in FIGS. 1, 2 and 3 respectively, demonstrate the stability of the solutions under storage conditions. Somewhat accelerated storage testing is often carried out by storing solutions at 37° C. The results of tests (see, e.g., FIGS. 1 and 3) demonstrated that both a 10% (w/v) solution of "native" ascorbic acid and a 30% (w/v) solution of pretreated ascorbic acid were stable after storage at 37° C. for one week.

[0030] As an example, containers having a 1 to 20% (w/v) concentration of a mixture of pretreated ascorbic acid in a 1:1 to 1:10 ratio, together with ascorbic acid formulated under more standard conditions (i.e., dissolved or added in solid form to a formulation at temperatures of about 20 to about 40°

C.—“native ascorbic acid”) were quite stable when shipped and/or stored under adverse conditions, or even when heated. The stability of such formulations was enhanced in comparison to conventional low pH formulations containing untreated ascorbic acid, e.g., low pH creams containing 10% (w/v) untreated ascorbic acid. It is postulated that the observed stability of the present compositions is afforded by an equilibrium reaction between ascorbic acid and monhydroascorbic acid that maintains a stable solution of ascorbic acid.

[0031] The present compositions generally also include a non-toxic zinc salt. The zinc salt may be a water-soluble zinc salt such as zinc sulfate. The zinc salt is generally present in about 0.5 to about 5.0% (w/v). Very effective results can typically be obtained with compositions that include no more than about 3.0% (w/v) zinc salt. For example, a number of present compositions are commonly formulated with about 0.5 to about 2.0% (w/v) zinc sulfate together with the other components described herein.

[0032] The composition of the present invention may further include one or more compounds capable of serving as a stimulant of protein synthesis and/or precursor to melanin synthesis. This component is generally present in about 1 to about 10% (w/v), and, for example, between about 3 to about 8% (w/v), based on the total composition. Typically, this component includes a tyrosine compound. As employed herein, a “tyrosine compound” is tyrosine or a compound that is capable of generating tyrosine upon chemical and/or biological transformation. Examples of suitable tyrosine compounds for use in the present compositions include tyrosine, N-acetyltyrosine, tyrosine ethyl ester hydrochloride, and tyrosine phosphate.

[0033] The present compositions may also include a compound which can function as an anti-inflammatory agent. Examples of suitable anti-inflammatory agents include anti-inflammatory sulfur-containing compounds and anti-inflammatory aminosugars. The sulfur-containing anti-inflammatory compound is typically a sulfur containing amino acid or related derivative such as cystine, cysteine, N-acetyl cysteine, glutathione, cysteamine, S-methylcysteine, methionine and the like. Examples of suitable anti-inflammatory aminosugars include glucosamine, mannosamine, N-acetylmannosamine, galactosamine, glucosamine-6-phosphate, N acetylglucosamine, N-acetylmannosamine, N-acetylgalactosamine and the like. For example, by adding D-glucosamine hydrochloride to the present compositions (in circa 5-20% (w/v)), cellular damage due to excess sun exposure can be minimized even if applied roughly 12 hours after exposure due to the anti-inflammatory effects of glucosamine in concert with ascorbic acid.

[0034] In addition to treating aged or damaged skin, the present invention further includes compositions and methods for treating inflammatory skin ailments such as rosacea, adult acne and like inflammations. Conventional treatment for rosacea includes cortisone therapy that involves continual use of cortisone, which causes connective tissue thinning. Conventional treatment for non-inflammatory rosacea (for example, red face due to surface blood vessels that become even more prominent after exposure to the sun or to the cold) includes metronizole, which is postulated to be an anti-inflammatory agent. Metronizole therapy requires continual use of metronizole while treatment is desired or necessary. Subjects that use cortisone or metronizole, which both require continual use, typically suffer rebound or recurrence of their

inflammation after discontinuing use of either agent. The present invention provides a treatment for inflammatory rosacea that does not have the undesirable effects of cortisone and that is effective for individuals that are resistant to cortisone treatment. The present invention provides the further benefit of decreasing or eliminating rebound of rosacea after discontinuance of use.

[0035] A composition of the present invention comprising, for example, approximately 14% ascorbic acid, 3.5% tyrosine, 1.5% zinc sulfate and 20% glucosamine in a topical cream, ointment or lotion is effective for the treatment of rosacea that is resistant to prior art treatments. An embodiment of the present invention for the treatment of inflammatory skin ailments such as rosacea includes a composition comprising ascorbic acid in the range of approximately 5% to 20% w/w in a pH range of approximately 3.7 to 4.1 and glucosamine in the range of approximately 10% to 25%. Additional embodiments may further comprise zinc sulfate and/or tyrosine.

[0036] A feature of the present invention is the combination of ascorbic acid and glucosamine. These two substances do not ordinarily combine well because the respective pHs are incompatible with each other. Mixing ascorbic acid with glucosamine produces a soured, brown mush unless at least a portion of the ascorbic acid is pretreated. For the combination of the present invention to mix stably, the use of pretreated ascorbic acid, as described above, adjusted to a pH in the range of approximately 3.8 to 4.0, produces a stable mixture of ascorbic acid and glucosamine. As described above, the pretreated ascorbic acid may be present in the range of approximately 10% to 50% of the total ascorbic acid in the formulation.

[0037] An embodiment of the present invention was used to treat a 49-year-old woman of Greek descent who had severe rosacea on her face for ten years and had used hydrocortisone constantly during this period to partially control the inflammation. Topical use of the present invention by the subject on the area of inflammation for a week resulted in complete remission of the rosacea.

[0038] A second subject was a 34-year-old woman who suffered from disfiguring rosacea on her face for 14 years. The second subject used hydrocortisone to partially control the inflammation, but even so she rarely went out in public and then only with heavy make-up. After failure of the cortisone treatment to provide adequate relief, the subject used an ascorbic acid, zinc sulfate and tyrosine formulation once a day. The ascorbic acid composition provided slight relief, but the subject's rosacea never cleared up adequately. The subject then used the same ascorbic acid formulation to which 20% (w/v) glucosamine had been added. After one week of using an ascorbic acid/glucosamine composition of the present invention, her complexion showed marked improvement. After three weeks of use, substantially all her blemishes disappeared and she no longer has to wear make-up.

[0039] FIG. 4 provides an exemplary depiction of the cosmetic and therapeutic benefits of the present invention. A subject used an embodiment of the present invention containing glucosamine for one week. Before using the embodiment (panel A), the subject suffered from disfiguring rosacea blemishes on her face. After one week of use (panel B), the subject shows marked improvement. In one week, the size of blemishes has decreased and the intensity of the rosacea has

abated, although some scarring (resulting from years of severe inflammatory lesions that had not responded to previous treatments) remains.

[0040] The ascorbic acid and tyrosine compound components of the present compositions may be formulated in part or whole in a neutralized or salt form. Acceptable amine salts include the acid addition salts (e.g., formed with a free amino group of a tyrosine compound) and may be formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, oxalic, tartaric, mandelic, and the like. Salts formed with the free carboxyl groups may also be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, histidine, procaine and the like. As noted elsewhere herein, since the present compositions have a pH of about 3.5 or above (and typically at least about 3.7) the ascorbic acid is typically at least partially present in the form of ascorbate salt(s), or possibly as an equilibrium reaction between ascorbic acid and monohydroascorbic acid. Commonly, the pH of the composition is adjusted to the desired value by adding sufficient base, such as sodium hydroxide, potassium hydroxide and/or ammonium hydroxide, to achieve the desired value. In such situations, the ascorbate would exist at least in part in the form of sodium hydroxide, potassium and/or ammonium ascorbate.

[0041] The water used for preparing the compositions of the present invention may be distilled and/or deionized, but any water may be used that does not contain contaminants that would affect the stability of the ascorbic acid present in the composition. For example, the presence of certain metal ions such as copper and iron salts, is known to affect the stability of ascorbic acid. The effects of water of varying purity on ascorbic acid stability is discussed in Meucci, et al., "Ascorbic Acid Stability in Aqueous Solutions," *Acta Vitaminol. Enzymol.* 7(34): 147-54 (1985), the disclosure of which is incorporated herein by reference.

[0042] The present compositions typically also include a pharmaceutically acceptable carrier. Carriers for topical application useful in practicing the invention include, but are not limited to, alkylene glycols, or alkylene glycols in combination with one or more derivatives of hydroxyalkylcellulose. In one illustrative embodiment, the alkylene glycol is propylene glycol and the hydroxyalkylcellulose is hydroxypropylcellulose. When a combination of alkylene glycol and hydroxyalkylcellulose is used, a useful ratio of alkylene glycol to hydroxyalkylcellulose is from about 30:1 to 5:1.

[0043] Without limitation, other carriers known to those skilled in the art that are compatible with water and are biologically or pharmaceutically acceptable are expected to provide equivalent compositions within the scope of this invention. For example, alcohols such as ethanol and propanol, glycols such as butylene or hexylene glycol, and polyols such as sorbitol or glycerol may be suitably employed. Other examples of suitable carriers include polyethylene or polypropylene glycols. Also contemplated as carriers for use in the present compositions are biologically acceptable hydroxyalkylcelluloses.

[0044] The phrase "pharmaceutically acceptable" refers to molecular entities and compositions that do not produce an allergic or similar untoward reaction when administered to a human. The pharmaceutically acceptable carriers and additives employed in the present compositions are compatible

with at least one formulation of the ascorbic acid/ascorbate mixture, tyrosine compound and zinc salt-containing compositions as described herein.

[0045] Amino acids employed in the present compositions will generally be in the left-handed chiral form of the amino acid (i.e., L-amino acid(s)). The amino acids should be as pyrogen free as possible and should meet sterility, pyrogenicity, general safety and purity standards as required by FDA Office of Drug standards. The amino acids may even act as buffers for the present solutions or may even be used to adjust the pH of the solution to above 3.5.

[0046] Illustrative examples of the present compositions can be produced as follows. The appropriate amounts of the acid forms of native and untreated ascorbic acid are mixed and/or dissolved in water. A water soluble, non-toxic zinc salt is then added and the mixture is mixed (via stirring or agitation) until the zinc salt has dissolved. Other components, such as a tyrosine compound and/or anti-inflammatory compounds are then added if desired.

[0047] After the other ingredients have been added to the solution, the pH is adjusted by adding an appropriate amount of a base such as sodium hydroxide or sodium carbonate to produce a pH of about 3.8 to about 4.0. The resulting solution can be employed as a topical composition in this form (i.e., a "serum") or may be used to produce any of a variety of conventional formulations well known to those skilled in the art, e.g., as a cream, lotion or gel.

[0048] The present topical composition may be in the form of an aqueous solution (i.e., "serum") or blended into a tissue compatible vehicle, such as hydrophilic lotion-, ointment-, cream- or gel-based vehicle. Such vehicles are well known in the art and commercially available for formulation of active ingredients into a suitable form for topical application. Exemplary of such vehicles are the commercially available Derma-base and Unibase formulations.

[0049] The present compositions may include one or more of a variety of optional ingredients, such as coloring agents, opacifying agents and the like. The compositions may include, in addition to the components described hereinabove, other active ingredients, such as antibiotics, analgesics, anti-allergens and the like. The formulation is commonly applied to the skin as a lotion or cream to be rubbed on body tissue over the desired area. For optimum efficacy treatment in accordance with the presented method should be initiated as early as possible following exposure to sunlight or another radiation source or upon the occurrence of a rosacea outbreak, rash, dermatitis, adult acne or other inflammatory skin response. Generally, a composition of the present invention may be applied to the skin once or twice daily. As noted elsewhere herein, the present composition may be used to inhibit the effects of aging and/or photo damage on the skin, or to treat rosacea, rash, dermatitis or other inflammatory skin complaint.

[0050] Upon formulation, compositions will be administered in a manner compatible with the formulated dosage and in such amount and frequency as is therapeutically effective. The compositions of the present invention are easily administered in a variety of administration forms suitable for direct topical application on skin. Pharmaceutically acceptable forms of administration include, but are not limited to, lotions, ointments, foams, emollients, microsphere and other encapsulants including time-release encapsulants, patches including, e.g., transdermal patches, shampoos, skin or hair conditioners, pomades, sprays or aerosols, water-based solu-

tions, oil/water emulsifications, gels, sera, unguents, salves, soaps, waxes, paraffins, gums, creams, tonics, elixirs, embrocations, lenitives, liniments, medicaments, balms, balsams, palliatives and any combination of administration forms suitable for topical application on skin.

[0051] For topical-administration in an aqueous solution, for example, the ascorbic acid/ascorbate mixture, tyrosine compound and zinc salt containing compositions of the present invention, including those embodiments containing glucosamine or other anti-inflammatory aminosugar, may be used directly on the skin without any toxic effects to the animal or patient. Alternatively, the ascorbic acid/ascorbate mixture, tyrosine compound and zinc salt containing compositions identified herein, including those containing glucosamine or other anti-inflammatory aminosugar, may be dissolved or resuspended in a suitable buffer prior to mixing, if necessary. Liquid diluents may first be rendered isotonic with sufficient saline or glucose solutions.

[0052] The present aqueous solutions are especially suitable for topical administration. As discussed above, however, other ascorbic acid-based formulations, including those containing glucosamine or other anti-inflammatory aminosugar, may also be used quite effectively. Some variation in dosage will necessarily occur depending on the condition of the subject being treated. The person responsible for administration will, in any event, determine the appropriate dose for the individual subject. Moreover, for human administration, preparations should meet sterility, pyrogenicity, general safety and purity standards as required by FDA Office of Biologics standards.

[0053] The topical compositions of the present invention may be included in lotions and creams, which may be a dermatologically acceptable emollient. Such compositions may contain from about 2% to about 50% of the emollient. An "emollient" as defined herein is a material useful for the prevention or relief of dryness, as well as for the protection of the skin. Many suitable emollients are known and may be used with the present invention, see e.g., Sagarin, *Cosmetics, Science and Technology*, 2nd Edition, Vol. 1, pp. 32-43 (1972), relevant portions incorporated herein by reference. A material suitable as an emollient is, e.g., glycerin. Glycerin may be used in an amount of from or about 0.001 to or about 20%, from or about 0.01 to or about 10, or even from about 0.1 to or about 5%, e.g., 3%.

[0054] Lotions and creams may also include a solution carrier system and one or more emollients. Lotions typically include from about 1% to about 20%, from about 5% to about 10%, of emollient; from about 50% to about 90%, from about 60% to about 80%, water; and other ingredients in the above described amounts. A cream includes typically from about 5% to about 50%, from about 10% to about 20%, of emollient; from about 45% to about 85%, from about 50% to about 75%, water; and other ingredients in the above described amounts.

[0055] Ointments of the present invention may include a simple carrier base of animal or vegetable oils or semi-solid hydrocarbons (oleaginous); absorption ointment bases which absorb water to form emulsions; or water soluble carriers, e.g., a water soluble solution carrier. Ointments may further include a thickening agent, such as described in Sagarin, *Cosmetics, Science and Technology*, 2nd Edition, Vol. 1, pp. 72-73 (1972), incorporated herein by reference, and/or an emollient. For example, an ointment may include from about

2% to about 10% of an emollient; from about 0.1% to about 2% of a thickening agent; and other ingredients in the above described amounts.

[0056] Compositions of this invention useful for cleansing ("cleansers") may be formulated with a suitable carrier, e.g., as described above, and contain, in addition to the ingredients in the amounts described above, from about 1% to about 90%, or from about 5% to about 10%, of a dermatologically acceptable surfactant. Surfactants may be selected from anionic, nonionic, zwitterionic, amphoteric and ampholytic surfactants, as well as mixtures of these surfactants. Such surfactants are well known to those skilled in the detergency art. Examples of surfactants include isoceteth-20, sodium methyl cocoyl taurate, sodium methyl oleoyl taurate, and sodium lauryl sulfate, see e.g., U.S. Pat. No. 4,800,197, to Kowcz et al., issued Jan. 24, 1989, relevant portions incorporated herein by reference, for exemplary surfactants. Examples of a broad variety of additional surfactants are described in McCutcheon's *Detergents and Emulsifiers*, North American Edition (1986), published by Allured Publishing Corporation, relevant portions incorporated herein by reference. The cleansing compositions may contain, at their art-established levels, other materials that are used conventionally in cleansing compositions.

[0057] The physical form of the cleansing, cream, lotion and other compositions is not critical. The compositions may be, for example, formulated as toilet bars, liquids, shampoos, bath gels, hair conditioners, hair tonics, pastes, or mousses. Toilet bars may be provided as a cleansing agent most commonly used to wash the skin, e.g., the face or scalp. Cleansing compositions that rinse-off, such as shampoos, may require a delivery system adequate to deposit sufficient levels of actives on the skin and scalp. One such delivery system involves the use of insoluble complexes. For a more complete disclosure of such delivery systems, see U.S. Pat. No. 4,835,148, Barford et al., issued May 30, 1989, relevant portions incorporated herein by reference.

[0058] The present invention may also be formulated as a foundation. As used herein, the term "foundation" refers to a liquid, semi-liquid, semi-solid, or solid skin cosmetic that includes, but is not limited to lotions, creams, gels, pastes, cakes, and the like. Typically, foundations are used over a large area of the skin, such as over the face, to provide a particular look. Foundations are used typically to provide an adherent base for color cosmetics such as rouge, blusher, powder and the like, and tend to hide skin imperfections and impart a smooth, even appearance to the skin.

[0059] Foundations will find particular usefulness in the present invention when used to not only reduce the level of inflammation associated with skin conditions such as rosacea, adult acne and the like, but also provide a make-up that will cover the treatment areas until the skin returns to its pre-inflammatory state. Foundations may include a dermatologically acceptable carrier for the formulations of the present invention, e.g., oils, colorants, pigments, emollients, fragrances, waxes, stabilizers and the like.

[0060] The compositions of the present invention may further include a non-toxic zinc salt. Zinc salts for use with the present invention may include zinc acetate, zinc acetate hydrates, zinc aluminum oxide complexes, zinc bromate, zinc bromide, zinc carbonates, zinc chloride hydrates, zinc chloride, zinc diamine dichloride, zinc citrate, zinc chromate, zinc dichromate, zinc diphosphate, zinc hexacyanofluoride ferrate (II), zinc fluoride, zinc fluoride hydrates, zinc formate, zinc

formate hydrates, zinc hydroxide, zinc iodate, zinc iodide, zinc iron oxide complexes, zinc nitrate hydrates, zinc nitride, zinc oxalate hydrates, zinc oxides, zinc perchlorate, zinc permanganate hydrates, zinc peroxide, zinc p-phenolsulfonate hydrates, zinc phosphate, zinc phosphate hydrates, zinc phosphide, zinc propionate, zinc selenate hydrates, zinc selenide, zinc silicates, zinc silicon oxide water complexes, zinc hexafluorosilicate hydrates, zinc stearate, zinc sulfate, zinc sulfate hydrates, zinc sulfide, zinc sulfite, zinc telluride, zinc thiocyanate, zinc (II) salts of N-acetyl L-cysteine, and mixtures thereof. Zinc salts may also include zinc citrate, zinc oxide, zinc chloride, zinc acetate, zinc stearate, zinc sulfate, and mixtures thereof, with Zinc citrate being particularly useful.

[0061] The compositions of the present invention may also include one or more humectants or moisturizers. A variety of these materials may be used at amount ranging from about 0.1% to about 20%, from about 0.5% to about 15%, and from about 1% to about 10%. Examples of humectants include materials selected from the group consisting of guanidine; glycolic acid and glycolate salts (e.g., ammonium and quaternary alkyl ammonium); lactic acid and lactate salts (e.g., ammonium and quaternary alkyl ammonium); aloe vera in any of its variety of forms (e.g., aloe vera gel); polyhydroxy alcohols such as sorbitol, glycerol, hexanetriol, propylene glycol, butylene glycol, hexylene glycol and the like; polyethylene glycols; sugars and starches; sugar and starch derivatives (e.g., alkoxyated glucose); hyaluronic acid; lactamide monoethanolamine; acetamide monoethanolamine; and mixtures thereof.

[0062] A particular humectant for use herein is glycerol. Other materials that may be used in conjunction with the present invention include: almond meal, alumina, aluminum oxide, aluminum silicate, apricot seed powder, attapulgite, barley flour, bismuth oxychloride, boron nitride, calcium carbonate, calcium phosphate, calcium pyrophosphate, calcium sulfate, cellulose, chalk, chitin, clay, corn cob meal, corn cob powder, corn flour, corn meal, corn starch, diatomaceous earth, dicalcium phosphate, dicalcium phosphate dihydrate, fullers earth, hydrated silica, hydroxyapatite, iron oxide, jojoba seed powder, kaolin, magnesium trisilicate, mica, microcrystalline cellulose, montmorillonite, oat bran, oat flour, oatmeal, peach pit powder, pecan shell powder, polybutylene, polyethylene, polyisobutylene, polymethylstyrene, polypropylene, polystyrene, polyurethane, nylon, teflon (i.e. polytetrafluoroethylene), polyhalogenated olefins, pumice rice bran, rye flour, cericite, silica, silk, sodium bicarbonate, sodium silicoaluminate, soy flour synthetic hectorite, talc, tin oxide, titanium dioxide, tricalcium phosphate, walnut shell powder, wheat bran, wheat flour, wheat starch, zirconium silicate, and mixtures thereof. Also useful are mixed polymers (e.g., copolymers terpolymers, etc.), such as polyethylene/polypropylene copolymer, polyethylene/propylene/isobutylene copolymer, polyethylene/styrene copolymer, and the like. Typically, the polymeric and mixed polymeric particles are treated via an oxidation process to destroy impurities and the like. The polymeric and mixed polymeric particles may also be crosslinked with a variety of common crosslinking agents. Examples of common cross-linking agents include: butadiene, di-vinyl benzene, methylenebisacrylamide, allyl ethers of sucrose, allyl ethers of pen-

taerythritol, and mixtures thereof. Other examples of useful particles include waxes and resins, such as: paraffins, carnauba wax, ozokerite wax, candellila wax, urea-formaldehyde resins and the like. When waxes and resins are used it is important that these materials are solids at ambient and skin temperatures.

[0063] The present invention also includes methods for delivering an effective amount of the composition of the present invention when applied to the skin. These compositions are useful for conditioning and treating e.g., inflamed, dry, aging and damaged skin.

[0064] The compositions of the present invention are useful for personal treatment and cleansing, especially of exposed areas of the skin such as face and neck. Typically, a suitable or effective amount of the composition is applied to the area to be treated. Alternatively, a suitable amount of the composition may be applied via intermediate application to a washcloth, sponge, pad, cotton ball or other application device. If necessary, the area to be treated may be cleaned or pre-moistened with water and/or a thin layer of an ointment, cream, oil and the like. It has been found that the compositions of the present invention may cause a mild irritation in patients with sensitive skins when applied directly. In those cases where skin irritation is a problem, the subject may apply a cream or other lotion to the area before using the composition of the present invention to reduce the level of skin irritation. Alternatively, the composition may be used or provided along with and wiped-off from the skin using a pad, cotton ball, tissue or other like device. The cleansing process is typically a two-step process involving application of the composition followed either by rinsing of the produce with water or wiping without the use of water. An effective amount of composition to be used will depend upon the needs of the individual.

[0065] While the making and using of various embodiments of the present invention are discussed in detail below, it should be appreciated that the present invention provides many applicable inventive concepts that can be embodied in a wide variety of specific contexts. The specific embodiments discussed herein are merely illustrative of specific ways to make and use the invention and do not delimit the scope of the invention. Various modifications and combinations of the illustrative embodiments, as well as other embodiments of the invention, will be apparent to persons skilled in the art upon reference to the description.

What is claimed is:

1. A method of treating rosacea or acne, the method comprising topically applying to the skin of a human afflicted with rosacea or acne an effective amount of a composition comprising:

at least 10% (w/v) ascorbic acid;
approximately 10% to 25% (w/v) glucosamine; and
water, wherein the composition has a pH of about 3.5 to about 4.1.

2. The method of claim 1, wherein the ascorbic acid is at about 15% to about 25% (w/v) of the composition.

3. The method of claim 1, wherein the ascorbic acid is at about 15% (w/v) of the composition.

4. The method of claim 1, wherein the water is distilled or deionized water.

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EXHIBIT B

United States Patent [19]

Murad

[11] **Patent Number:** **5,804,594**
 [45] **Date of Patent:** **Sep. 8, 1998**

- [54] **PHARMACEUTICAL COMPOSITIONS AND METHODS FOR IMPROVING WRINKLES AND OTHER SKIN CONDITIONS**
- [76] Inventor: **Howard Murad**, 4316 Marina City Dr., Marina del Rey, Calif. 90292
- [21] Appl. No.: **787,358**
- [22] Filed: **Jan. 22, 1997**
- [51] **Int. Cl.**⁶ **A61K 31/715**; A61K 31/34; A61K 31/19
- [52] **U.S. Cl.** **514/474**; 514/557; 514/801; 514/474; 514/62; 514/54; 424/417
- [58] **Field of Search** 514/54, 62, 474, 514/557, 801; 424/417

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[57] ABSTRACT

This application relates to a pharmaceutical composition for the prevention and treatment of skin conditions in a patient having a sugar compound that is converted to a glycosaminoglycan in the patient in an amount sufficient to thicken the skin, a primary antioxidant component in an amount sufficient to substantially inhibit the formation of collagenase and elastase, at least one amino acid component in an amount sufficient to assist in the thickening of the skin, and at least one transition metal component in an amount effective to bind collagen and elastic fibers and rebuild skin. In one preferred form, the composition further includes a catechin-based preparation, a glucosamine or a pharmaceutically acceptable salt or ester thereof, and a chondroitin or a pharmaceutically acceptable salt or ester thereof. In a more preferred form, the invention further includes a vitamin E source, a cysteine source, a vitamin B₃ source, quercetin dihydrate, pyridoxal 5 phosphate-Co B₆, a methionine source, and a vitamin A source. The invention further relates to a method for the prevention or treatment of skin conditions by administering the pharmaceutical composition in an amount therapeutically effective to modify the thickness of the skin to prevent or treat at least one skin condition.

19 Claims, No Drawings

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PHARMACEUTICAL COMPOSITIONS AND METHODS FOR IMPROVING WRINKLES AND OTHER SKIN CONDITIONS

TECHNICAL FIELD

This application relates to pharmaceutical compositions, as well as methods, to supplement collagen and elastic tissues and thicken the dermis for the treatment of wrinkles and other skin conditions.

BACKGROUND OF THE INVENTION

Human skin is a composite material of the epidermis and the dermis. The topmost part of the epidermis is the stratum corneum. This layer is the stiffest layer of the skin, as well as the one most affected by the surrounding environment. Below the stratum corneum is the internal portion of the epidermis. Below the epidermis, the topmost layer of the dermis is the papillary dermis, which is made of relatively loose connective tissues that define the micro-relief of the skin. The reticular dermis, disposed beneath the papillary dermis, is tight, connective tissue that is spatially organized. The reticular dermis is also associated with coarse wrinkles. At the bottom of the dermis lies the subcutaneous layer.

The principal functions of the skin include protection, excretion, secretion, absorption, thermoregulation, pigmentogenesis, accumulation, sensory perception, and regulation of immunological processes. These functions are detrimentally affected by the structural changes in the skin due to aging and excessive sun exposure. The physiological changes associated with skin aging include impairment of the barrier function and decreased turnover of epidermal cells, for example. [Cerimele, D., et al., *Br. J. Dermatol.*, 122 Suppl. 35, p. 13-20 (April 1990)].

The mechanical properties of the skin, such as elasticity, are controlled by the density and geometry of the network of collagen and elastic fiber tissue therein. Damaged collagen and elastin lose their contractile properties, resulting in skin wrinkling and skin surface roughness. As the skin ages or becomes unhealthy, it acquires sags, stretch marks, bumps, bruises or wrinkles, it roughens, and it has reduced ability to synthesize Vitamin D. Aged skin also becomes thinner and has a flattened dermoepidermal interface because of the alterations in collagen, elastin, and glycosaminoglycans. [Fenske, N. A., and Lober, C. W., *J. Am. Acad. Dermatol.*, 15:571-585 (October 1986); Montagna, W. and Carlisle, K., *Journal of Investigative Dermatol.*, 73(1):47-53 (1979)].

A variety of vitamins and minerals have in individually been administered to treat certain skin and other problems that occur when the patient has a deficiency of that vitamin or mineral. Vitamin A, for example, assists in the treatment of acne and to facilitate wound healing; vitamin C (ascorbic acid) assists in the prevention of skin bruising and wound healing; vitamin E is an antioxidant; and copper assists in the treatment of elastic tissue defects. [Neldner, K. H., *Amer. Acad. Derm. Annl. Mtg.*, Wash D.C., Dec. 6, 1993]. Topical use of vitamin C is also believed to ward off sun damage, reduce breakdown of connective tissues, and possibly promote collagen synthesis. [Dial, W., *Medical World News*, p. 12, March 1991]. Vitamin E is used topically as an anti-inflammatory agent, for enhancement of skin moisturization, for UV-ray protection of cells, and for retardation of premature skin aging.

Catechin-based preparations, including proanthanols and proanthocyanidins are powerful antioxidants. These compounds are found in flowers, plant leaves, and grape seeds, for example. [Lubell, A., *Cosmetic Dermatol.*, 9(7):58 & 60 (July 1996)].

N-Acetylglucosamine and glucosamine have been examined for use in the prevention and treatment of degenerative joint diseases and cartilage loss, and found to increase the glycosaminoglycans present in the cartilage to restore cartilage. [See Grevenstein, J., et al., *Acta Orthopaedia Belgica*, 57(2):157-161 (1991); Setnikar, I., *Drug Res.*, 36(4):720-733 (1986); Drovanti, A., et al., *Clin. Therap.*, 3(4):1-6 (1980)]. Glucosamine has also been examined in connection with arthritis [See, e.g., Murray, M. T.] and oral and injected glucosamine have been reported to be useful for arthrosic patients. [Tapadinhas, M. J., et al., *Pharmatherapeutica*, 3(3):157-168 (1982); D'Ambrosio, E., et al., *Pharmatherapeutica*, 2(8):504-508 (1981)].

The metabolism of glycosaminoglycans under the influence of herbal and other anti-inflammatory agents has been examined by measuring glycosaminoglycans in the skin, liver, kidney, and spleen after administration of several compounds. [Reddy, G. K., et al., *Biochem. Pharmacology*, 38(20):3527-3534 (1989)].

In addition to their individual use to supplement a deficiency in a patient, various of the above ingredients have been combined to form pharmaceuticals designed to prevent and treat certain cellular, skin, and other conditions. For example, U.S. Pat. No. 3,773,930 discloses a low residue, dietary composition having at least one amino acid and a quantity of non-amino acid derived caloric material sufficient to obviate the diarrhea problem of straight amino acid compositions. A flavoring material may also be included to render the composition more palatable.

U.S. Pat. No. 4,285,964 discloses a salt of (+)-catechin formed by reacting (+)-catechin with at least a basic amino acid, such as L-lysine and L-arginine; and a hydrosoluble double salt formed from the reaction product of (+)-catechin with a basic amino-acid, such as L-lysine and L-arginine, and another inorganic or organic acid. The patent further discloses methods of treating degenerative diseases of the connective tissue by topically administering the composition.

U.S. Pat. No. 4,414,202 discloses a composition for the treatment of skin wounds with a buffered salt solution having a pH between 6 to 7.8 and administering a starch hydrolysate compound, and preferably including alphaketoglutaric acid or alphaketoglutarate salts. Optional additives to the composition include ascorbic acid or salts thereof, ferrous salts, and glycine, L-Proline, and L-Lysine.

U.S. Pat. No. 4,424,232 discloses a topical composition for the treatment of herpes simplex, cold sores, lesions, and other painful skin conditions including L-lysine, gibberellic acid, and urea in an inert carrier having water. The composition may also include L-ascorbic acid, as well as methyl paraben, propyl paraben, or mixtures thereof.

U.S. Pat. No. 4,647,453 discloses a method and composition for treatment of tissue degenerative inflammatory disease in animals and humans by oral administration of ascorbic acid, bioavailable calcium, a precursor or stimulant of epinephrine or nor-epinephrine of tyrosine or phenylalanine, and an anti-inflammatory substance selected from anti-inflammatory sugars, amino sugars and biocompatible acid addition salts thereof, and anti-inflammatory amino acids, to promote connective tissue regrowth.

U.S. Pat. No. 5,198,465 discloses a composition for treating precursor deficiencies in the synthesis of collagen with proline, glycine, lysine, vitamin C, and one or more compounds selected from a-ketoglutaric acid, methionine, cysteine, cystine, valine, and pharmaceutically acceptable diluents and excipients.

U.S. Pat. Nos. 5,332,579 and 5,308,627 disclose a nutritional supplement to assist persons recovering from addiction by administering a variety of vitamins and minerals including enzyme activating substances such as magnesium and zinc; an enzyme co-factor that is a vitamin like various vitamin B complexes; an enzyme producer such as an amino acid like glutamic acid; an herbal antispasmodic substance like Valerian root; and vitamin C.

U.S. Pat. No. 5,415,875 discloses a method of suppressing formation of lipid peroxide and removing peroxide by applying to the skin a decomposed product of shell membrane and tocopherol and derivatives. Lysine, proline, Vitamin C, for examples, are listed among a vast genus of optional additives.

The above references, however, do not teach pharmaceutical compositions or methods for improving skin wrinkles along with other conditions, such as skin elasticity and softness. Thus, it is desired to find a pharmaceutical composition and a method for the prevention and treatment of wrinkles and other skin conditions. The present invention advantageously provides pharmaceutical compositions, as well as methods of treatment comprising the administration of such compositions, to repair skin for the prevention and treatment of wrinkles and other skin disorders.

SUMMARY OF THE INVENTION

The present invention relates to a pharmaceutical composition for the prevention and treatment of skin conditions in a patient having a sugar compound that is converted to a glycosaminoglycan in the patient in an amount sufficient to thicken the skin, a primary antioxidant component in an amount sufficient to substantially inhibit the activity of collagenase and elastase, at least one amino acid component in an amount sufficient to assist in the thickening of the skin, and at least one transition metal component in an amount effective to bind collagen and elastic fibers and rebuild skin.

In one embodiment, the sugar compound is present in about 5 to 50 weight percent, the primary antioxidant component is present in about 5 to 50 weight percent, the amino acid component is present in about 8 to 60 weight percent, and the transition metal component is present in about 0.5 to 15 weight percent of the composition. In another embodiment, the sugar compound includes an N-acetylglucosamine compound or salt or ester thereof, the primary antioxidant component includes an ascorbic acid component or salt or ester thereof, at least two amino acids selected from the group of proline, lysine, cysteine, and methionine are present, and at least two the transition metal components including zinc, manganese or copper, or mixtures thereof, are present. In yet another embodiment, the composition further includes a pharmaceutically acceptable carrier or excipient.

In a more preferred embodiment, at least three transition metal components are present, one of which is zinc monomethionine, one of which is manganese ascorbate, and one of which is copper sebacate, wherein the zinc is present in about 10 to 30 weight percent of the complex and the manganese is present in about 5 to 20 weight percent of the complex, and the copper is present in about 5 to 20 weight percent of the complex. In another preferred embodiment, the N-acetylglucosamine is present in about 5 to 30 weight percent, the ascorbic acid is present in about 5 to 50 weight percent, the amino acid comprises lysine and proline, wherein each is present in about 4 to 25 weight percent, and the zinc monomethionine and the manganese ascorbate are each present in about 1 to 10 weight percent and the copper sebacate is present in about 0.1 to 5 weight percent of the composition.

In one preferred embodiment of the invention, the composition further includes a catechin-based preparation, a glucosamine or a pharmaceutically acceptable salt or ester thereof, and a chondroitin or a pharmaceutically acceptable salt or ester thereof. In a more preferred embodiment, the catechin-based preparation is a proanthanol or proanthocyanidin, and the glucosamine and chondroitin are each a sulfate or succinate. In a most preferred embodiment, the proanthocyanidin is grape seed extract present in about 0.5 to 5 weight percent, the glucosamine is D-glucosamine sulfate present in about 3 to 17 weight percent, wherein the glucosamine is about 60 to 90 weight percent of the salt, and the chondroitin is chondroitin sulfate present in about 3 to 17 weight percent of the composition, wherein the chondroitin is preferably present as about 65 to 95 weight percent of the salt.

In another preferred embodiment, the composition further includes a vitamin E source, a cysteine source, a vitamin B₃ source, quercetin dihydrate, pyridoxal 5 phosphate-Co B₆, a methionine source, and a vitamin A source. In a more preferred embodiment, the vitamin E is D-alpha tocopheryl acid succinate present in about 1 to 15 weight percent, the vitamin B₃ is niacinamide present in about 0.5 to 15 weight percent, the vitamin A is vitamin A palmitate present in about 0.1 to 5 weight percent, the cysteine is N-acetyl cysteine present in about 1 to 10 weight percent, the methionine is preferably L-selenomethionine present in about 0.1 to 5 weight percent, the quercetin dihydrate is present in about 0.5 to 15 weight percent, and the pyridoxal 5 phosphate-Co B₆ is present in about 0.1 to 5 weight percent of the composition.

The invention further relates to a method for the prevention or treatment of skin conditions, wherein the skin has a thickness of dermis and collagen, which includes administering the pharmaceutical composition above in an amount therapeutically effective to modify the thickness of the skin to prevent or treat at least one skin condition.

In one embodiment according to the invention, the skin condition treated is at least one of wrinkles, fine lines, thinning, reduced skin elasticity, reduced skin moisture, spider veins, senile purpura, sun damaged skin, aging skin, or rough skin. In another embodiment, the composition is administered orally. In a preferred embodiment, the composition is administered as a tablet or capsule having about 1 mg to 2,000 mg of composition. In a more preferred embodiment, the tablet or capsule has about 200 mg to 1,600 mg of composition, and in a most preferred embodiment, the tablet or capsule has about 600 mg to 1,000 mg of composition.

In another embodiment, the composition is administered in conjunction with concurrent or subsequent treatment by at least one additional pharmaceutical composition for the prevention or treatment of a skin condition.

The ranges of the components of the pharmaceutical composition may vary, but the active ingredients should be understood to add to 100 weight percent of the active pharmaceutical composition.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

A formulation for the reduction of wrinkles and the improvement of other skin conditions, such as increased skin elasticity and skin softness, has now been discovered. Moreover, the prevention or treatment of unhealthy skin, such as aged skin or skin overexposed to sunlight, may advantageously be accomplished by the administration of

the pharmaceutical composition of the present invention to a human in need of treatment. The pharmaceutical composition includes the combination of a number of different components which interact to provide the desired improvements to the skin.

The advantageous pharmaceutical composition of the present invention prevents and improves skin conditions by using a sufficient amount of at least one sugar compound which is converted into glycosaminoglycans in the bloodstream, in combination with other ingredients disclosed herein to assist in thickening the dermis and supplementing collagen and elastic tissues. A thicker dermis desirably reduces the wrinkling and lines that occur when areas of the skin become thin. Various amino acids such as lysine, proline and cysteine assist in the thickening of the dermis, supplementing of collagen and elastic tissues and, consequently, reduction of wrinkles and other skin conditions. Additionally, antioxidants, such as vitamin C, inhibit collagenase and elastase, enzymes that break down collagen and elastic tissues. These antioxidants assist in the prevention of additional wrinkles and facilitate the healing of skin tissues. Finally, transition metal components are included to bind collagen fibers and inhibit elastase, an enzyme that also breaks down collagen and elastic tissue.

The composition preferably contains at least one sugar compound, and more preferably just one sugar compound, present in about 5 to 50 weight percent, preferably about 10 to 40 weight percent, and more preferably about 15 to 30 weight percent of the composition. The primary antioxidant component is preferably present in an amount of about 5 to 50 weight percent, more preferably about 10 to 40 weight percent, and most preferably about 15 to 30 weight percent of the composition. The amino acid component is preferably present in about 8 to 60 weight percent, more preferably about 15 to 50 weight percent, most preferably about 20 to 40 weight percent of the composition. The transition metal component is preferably present in about 0.5 to 15 weight percent, more preferably present in about 2 to 12 weight percent, and most preferably present in about 5 to 10 weight percent of the composition.

The first component of the composition is any sugar compound that is converted to glycosaminoglycans in the human bloodstream. Typically, this would be an N-acetylglucosamine compound, or a pharmaceutically acceptable salt or ester thereof. The N-acetylglucosamine component may be N-acetylglucosamine or any pharmaceutically acceptable salt or ester thereof, but more preferably is the N-acetylglucosamine only. This component must be present in sufficient quantity in the pharmaceutical composition to promote thickening of the dermis. The N-acetylglucosamine is present in about 5 to 30 weight percent, preferably 8 to 27 weight percent, and more preferably 12 to 24 weight percent of the pharmaceutical composition. A unit dose of N-acetylglucosamine is typically about 40 mg to 250 mg, preferably about 60 to 200, and more preferably about 100 mg to 200 mg.

The pharmaceutical composition includes a primary antioxidant, which typically is a vitamin C source and preferably is ascorbic acid, or a pharmaceutically acceptable salt or ester thereof, and more preferably is ascorbyl palmitate, dipalmitate L-ascorbate, sodium L-ascorbate-2-sulfate, or an ascorbic salt, such as sodium, potassium, or calcium ascorbate, or mixtures thereof. When oral formulations of the pharmaceutical composition are used, it is preferred that a non-acidic form of vitamin C be used to reduce the stomach irritation that may occur when using an acidic form. The vitamin C source is present in the phar-

maceutical composition in about 5 to 50 weight percent, preferably about 7 to 40 weight percent, and more preferably about 10 to 25 weight percent. A unit dose of this primary vitamin C source is typically about 40 mg to 400 mg, preferably about 60 mg to 300 mg, and more preferably about 80 to 150 mg. Vitamin C is also approved by the FDA and has wide consumer acceptance, so that it can be used in amounts as high as 10,000 mg, if desired.

The pharmaceutical composition also includes at least one amino acid to assist in thickening the skin. Preferably two or more amino acids are used in combination. Either the L- or D- forms of amino acids are acceptable. Lysine and proline are the most preferred amino acids and are advantageously used in combination. Cysteine, methionine or other amino acids can also be used, if desired. The amino acids may be included in a soluble form such as the hydrochloride, i.e., L-Lysine hydrochloride. The amino acids are present in an amount of about 2 to 25 weight percent each, preferably about 4 to 20 weight percent each, and more preferably about 6 to 15 weight percent each. A unit dose for each amino acid is typically about 35 mg to 200 mg each, preferably about 50 mg to 150 mg each, and more preferably about 70 mg to 120 mg in the pharmaceutical composition. Additional useful forms of amino acid include the following: a cysteine source, preferably N-acetyl cysteine, can be present in an amount of about 1 to 10 weight percent, preferably about 2 to 8 weight percent, and more preferably about 3 to 6 weight percent of the pharmaceutical composition. A methionine source, preferably L-selenomethionine, can be present in an amount of about 0.1 to 5 weight percent, preferably 0.2 to 3 weight percent, and more preferably 0.3 to 1 weight percent of the composition, wherein the selenium component is between about 0.1 to 3 weight percent of the methionine source.

One or more transition metal compounds are included in an amount effective to bind collagen and elastic tissue to rebuild the skin. Certain transition metal compounds inhibit the elastase enzyme to inhibit collagen and elastic tissue breakdown. Preferred transition metals include zinc, manganese and copper, with combinations thereof being most preferred.

A zinc component can be added to assist in binding collagen and elastic fibers, which both assists in the prevention of wrinkles and the rebuilding of wrinkled skin. The zinc component may be any zinc compound or pharmaceutically acceptable salt thereof, but more preferably is a zinc complexed with an amino acid, and most preferably is zinc monomethionine, wherein the zinc is typically present in about 10 to 30 weight percent of the complex. The zinc component is present in about 1 to 10 weight percent, more preferably about 2 to 7 weight percent, and most preferably about 3 to 5 weight percent of the pharmaceutical composition.

A manganese component can also be added to assist in binding collagen and elastic fibers. The manganese component may be any manganese compound or pharmaceutically acceptable salt thereof, but more preferably is a manganese component which is at least partially complexed with a vitamin C source, and most preferably is manganese ascorbate or manganese ascorbic acid, wherein the manganese is typically present in about 5 to 20 weight percent of the complex. When complexed with vitamin C, this vitamin C source may be included in the overall percentage of vitamin C in the pharmaceutical composition. The manganese component is present in about 1 to 10 weight percent, more preferably about 2 to 7 weight percent, and most preferably about 2.5 to 4 weight percent of the pharmaceutical composition.

A copper component is preferably also included in the pharmaceutical composition, and may be any copper compound or pharmaceutically acceptable salt thereof, but preferably is copper sebacate, wherein the copper is typically present in about 5 to 20 weight percent of the copper sebacate. The copper component also inhibits elastase and is present in about 0.1 to 5 weight percent, preferably about 0.2 to 3 weight percent, and more preferably about 0.3 to 1 weight percent of the pharmaceutical composition. A unit dose of the pharmaceutical composition may include about 1 mg to 40 mg, preferably about 2 mg to 25 mg, and more preferably about 2.5 mg to 10 mg.

In a preferred form of the invention, the pharmaceutical composition further includes a catechin-based preparation, such as a proanthanol or proanthocyanidin, along with glucosamine or a pharmaceutically acceptable salt or ester thereof, and chondroitin or a pharmaceutically acceptable salt or ester thereof.

The catechin-based preparation, similar to vitamin C, inhibits elastase and collagenase, which is another enzyme that attacks elastic tissue and collagen. The catechin-based preparation is preferably a proanthanol or proanthocyanidin, more preferably a proanthocyanidin, and most preferably grape seed extract. These compounds are considered to be secondary antioxidants, because they are present in lesser amounts than the primary antioxidant. The catechin-based preparation is present in about 0.5 to 5 weight percent, more preferably about 0.6 to 3 weight percent, and most preferably about 0.7 to 2 weight percent of the pharmaceutical composition.

The glucosamine or a pharmaceutically acceptable salt or ester thereof, and the chondroitin or a pharmaceutically acceptable salt or ester thereof are each present in about 3 to 17 weight percent, preferably about 4 to 12 weight percent each, and more preferably about 5 to 8 weight percent each of the pharmaceutical composition. The glucosamine component preferably is present as a sulfate or succinate, and more preferably is D-glucosamine sulfate, wherein the glucosamine is preferably present as about 60 to 90 weight percent of the salt. The glucosamine content of this component contributes to the formation of glycosaminoglycans in the skin. The chondroitin component preferably is present as a sulfate or succinate, and more preferably is chondroitin sulfate, wherein the chondroitin is preferably present as about 65 to 95 weight percent of the salt.

In a more preferred form, several optional additives are included in the pharmaceutical composition, such as a vitamin E source, a vitamin B₃ source, quercetin powder, pyridoxal 5 phosphate-Co B₆, and a vitamin A source. The vitamin E preferably is a sulfate or succinate vitamin E complex, and more preferably is D-alpha tocopheryl acid succinate. The vitamin E source is present in about 1 to 15 weight percent, preferably about 2 to 12 weight percent, and more preferably about 3 to 10 weight percent of the composition. In any event, no more than 1,500 IU should be ingested per day, as Vitamin E becomes toxic at higher doses. The vitamin B₃ source preferably is niacinamide, and the source is present in about 0.5 to 15 weight percent, preferably about 1 to 12 weight percent, and more preferably about 1.5 to 10 weight percent of the composition. The vitamin A source preferably is vitamin A palmitate, and the source is present in about 0.1 to 5 weight percent, preferably 0.2 to 3 weight percent, and more preferably 0.3 to 1 weight percent of the composition. In the more preferred form, the amount of vitamin A dosage is about 500,000 IU / gram per unit dose. Vitamin A is toxic at high levels, such that no more than 400,000 IU should be cumulatively ingested per day for greater than six months.

The quercetin powder is quercetin dihydrate, which is typically present in about 0.5 to 15 weight percent, preferably about 1 to 12 weight percent, and more preferably about 1.5 to 10 weight percent of the composition. The pyridoxal 5 phosphate-Co B₆, also known as P-5-P monohydrate, is typically present in about 0.1 to 5 weight percent, preferably 0.2 to 3 weight percent, and more preferably 0.3 to 1 weight percent of the composition.

The phrase "therapeutically effective amount" means that amount of the pharmaceutical composition that provides a therapeutic benefit in the treatment, prevention, or management of skin wrinkles and other skin conditions.

The magnitude of a prophylactic or therapeutic dose of the composition in the acute or chronic management of wrinkles will vary with the severity of the condition to be treated and the route of administration. The dose, and perhaps the dose frequency, will also vary according to the age, body weight, and response of the individual patient. In general, the total daily dose range, for the conditions described herein, is from about 10 mg to about 20,000 mg administered in single or divided doses orally, topically, transdermally, or locally by inhalation. For example, a preferred oral daily dose range should be from about 10 mg to 20,000 mg, more preferably about 2,000 mg to 16,000 mg, and most preferably about 6,000 mg to 10,000 mg of the active components (i.e., excluding excipients and carriers).

It is further recommended that children, patients aged over 65 years, and those with impaired renal or hepatic function initially receive low doses, and that they then be titrated based on individual response(s) or blood level(s). It may be necessary to use dosages outside these ranges in some cases, as will be apparent to those of ordinary skill in the art. Further, it is noted that the clinician or treating physician will know how and when to interrupt, adjust, or terminate therapy in conjunction with individual patient response.

The term "unit dose" is meant to describe a single dose, although a unit dose may be divided, if desired. About 1 to 10 unit doses of the present invention are typically administered per day, preferably about 2 to 6 doses per day, and more preferably about 4 doses per day.

Although any suitable route of administration may be employed for providing the patient with an effective dosage of the composition according to the methods of the present invention, oral administration is preferred. Suitable routes include, for example, oral, rectal, parenteral, intravenous, topical, transdermal, subcutaneous, intramuscular, and like forms of administration may be employed. Suitable dosage forms include tablets, troches, dispersions, suspensions, solutions, capsules, patches, suppositories, and the like, although oral dosage forms are preferred.

The pharmaceutical compositions used in the methods of the present invention include the active ingredients described above, and may also contain pharmaceutically acceptable carriers, excipients and the like, and optionally, other therapeutic ingredients.

The term "pharmaceutically acceptable salt" refers to a salt prepared from pharmaceutically acceptable non-toxic acids or bases including inorganic or organic acids. Examples of such inorganic acids are hydrochloric, hydrobromic, hydroiodic, sulfuric, and phosphoric. Appropriate organic acids may be selected, for example, from aliphatic, aromatic, carboxylic and sulfonic classes of organic acids, examples of which are formic, acetic, propionic, succinic, glycolic, glucuronic, maleic, furoic, glutamic, benzoic, anthranilic, salicylic, phenylacetic,

mandelic, embonic (pamoic), methanesulfonic, ethanesulfonic, pantothenic, benzenesulfonic, stearic, sulfanilic, algenic, and galacturonic. Examples of such inorganic bases, for potential salt formation with the sulfate or phosphate compounds of the invention, include metallic salts made from aluminum, calcium, lithium, magnesium, potassium, sodium, and zinc. Appropriate organic bases may be selected, for example, from N,N-dibenzylethylenediamine, chloroprocaine, choline, diethanolamine, ethylenediamine, meglumaine (N-methylglucamine), and procaine.

The compositions for use in the methods of the present invention include compositions such as suspensions, solutions and elixirs; aerosols; or carriers such as starches, sugars, microcrystalline cellulose, diluents, granulating agents, lubricants, binders, disintegrating agents, and the like, in the case of oral solid preparations (such as powders, capsules, and tablets), with the oral solid preparations being preferred over the oral liquid preparations. The most preferred oral solid preparations are tablets.

Because of their ease of administration, tablets and capsules represent the most advantageous oral dosage unit form, in which case solid pharmaceutical carriers are employed. If desired, tablets may be coated by standard aqueous or nonaqueous techniques.

In addition to the common dosage forms set out above, the compound for use in the methods of the present invention may also be administered by controlled release means and/or delivery devices such as those described in U.S. Pat. Nos. 3,845,770; 3,916,899; 3,536,809; 3,598,123; and 4,008,719, the disclosures of which are hereby incorporated by reference.

Pharmaceutical compositions for use in the methods of the present invention suitable for oral administration may be presented as discrete units such as capsules, cachets, or tablets, or aerosol sprays, each containing a predetermined amount of the active ingredient, as a powder or granules, as creams, pastes, gels, or ointments, or as a solution or a suspension in an aqueous liquid, a non-aqueous liquid, an oil-in-water emulsion, or a water-in-oil liquid emulsion. Such compositions may be prepared by any of the methods of pharmacy, but all methods include the step of bringing into association the carrier with the active ingredient which constitutes one or more necessary ingredients. In general, the compositions are prepared by uniformly and intimately admixing the active ingredient with liquid carriers or finely divided solid carriers or both, and then, if necessary, shaping the product into the desired presentation.

For example, a tablet may be prepared by compression or molding, optionally, with one or more accessory ingredients. Compressed tablets may be prepared by compressing in a suitable machine the active ingredient in a free-flowing form such as powder or granules, optionally mixed with a binder, lubricant, inert diluent, surface active or dispersing agent. Molded tablets may be made by molding, in a suitable machine, a mixture of the powdered compound moistened with an inert liquid diluent. Desirably, each unit dose, i.e., tablet, cachet or capsule, contains from about 1 mg to 2,000 mg of the active ingredient, preferably about 200 mg to 1,600 mg, and more preferably about 600 mg to 1,000 mg of the composition.

EXAMPLES

The invention is further defined by reference to the following examples describing in detail the preparation of the compound and the compositions used in the methods of

the present invention, as well as their utility. The examples are representative, and they should not be construed to limit the scope of the invention.

Example 1: Capsules

A large number of unit capsules are prepared by filling standard two-piece hard gelatin capsules each with the desired amount of powdered active ingredient as described above, 150 milligrams of lactose, 50 milligrams of cellulose, and 6 milligrams magnesium stearate.

Example 2: Soft Gelatin Capsules

A mixture of active ingredient in a digestible oil such as soybean oil, lecithin, cottonseed oil or olive oil is prepared and injected by means of a positive displacement pump into gelatin to form soft gelatin capsules containing the desired amount of the active ingredient. The capsules are washed and dried for packaging.

Example 3: Tablets

A large number of tablets were prepared by conventional procedures so that the dosage unit included: the desired amount of active ingredient as described herein, 50 milligrams of red beet root powder, 12 milligrams of stearic acid, 10.95 milligrams of sorbitol, 3 milligrams of acdisol, 1 milligram of magnesium stearate, and 1 milligram of syloid. Appropriate coatings may be applied to increase palatability or delay absorption. A specific therapeutic formulation of the pharmaceutical composition described herein is set forth in the table below:

Ingredient	Weight Percent (% w/w)	Amount (mg)	Chemical or Scientific Name (if different)
N-Acetylglucosamine	17.1	140	N-Acetyl D-Glucosamine
Vitamin C (81.2% Ascorbic Acid)	15	123.2	
L-Lysine (80%)	12.2	100	L-Lysine hydrochloride
L-Proline	11	90	
D-Glucosamine Sulfate (75%)	6.5	53.3	
Chondroitin Sulfate (80%)	6.1	50	
Vitamin E Succinate	4.3	39.7	D- α tocopheryl acid succinate
Zinc monomethionine (20%)	3.7	30	Zinc DL-methionine
N-Acetyl Cysteine	3.7	30	
Manganese Ascorbate (13% Mn)	2.8	23.1	
Vitamin B ₃	2.4	20	Niacinamide
Niacinamide	2.4	20	
Quercetin Powder	2.4	20	Quercetin dihydrate
Grape Seed Extract	0.9	7.5	Proanthocyanidin
Pyridoxal 5 Phosphate-Co B ₆	0.6	5	P-5-P monohydrate
Selenomethionine (0.5%)	0.5	4	L-selenomethionine
Vitamin A Palmitate (500,000 IU/GR)	0.5	4	
Copper Sebacate (14%)	0.4	2.9	
Red beet root powder	6.1	50	Beta vulgaris rubra
Stearic acid	1.5	12	
Sorbitol	1.3	11	
Acdisol	0.4	3	Microcrystalline cellulose

-continued

Ingredient	Weight Percent (% w/w)	Amount (mg)	Chemical or Scientific Name (if different)
Coconut oil	0.1	1	Magnesium stearate
Syloid	0.1	1	Silicon dioxide (amorphous)
Total	820.7	100	

These tablets are an example of a preferred embodiment of a unit dose according to the present invention.

number of wrinkles; (b) total area of wrinkles; (c) total length of wrinkles; (d) mean length of wrinkles; and (e) mean depth of wrinkles. The type of wrinkles was determined on the basis of depth, length, and area.

As indicated in Table I below, the number of wrinkles were significantly reduced by 34 percent ($p < 0.01$) and the number of fine lines by 34 percent ($p < 0.06$) as a result of 5 weeks using the test material.

TABLE I

	Number of Wrinkles and Fine Lines							
	Number of Wrinkles				Number of Fine Lines			
	Mid-Baseline		Final-Baseline		Mid-Baseline		Final-Baseline	
	Control	Treated	Control	Treated	Control	Treated	Control	Treated
Average	-3	-7	-3	-15	-5	-4	-6	-12
Standard Deviation	9	13	13	12	6	10	14	10
p value	$p < 0.41$		$p < 0.01$		$p < 0.96$		$p < 0.06$	
% Difference from Baseline	-11%	-19%	-6%	-40%	-14%	-24%	-9%	-43%
Total % Difference (T - C)	-8%		-34%		-10%		-34%	

T = Treated
C = Control

Examples 4-7: Testing of the Product

The tablets of Example 3 were administered to test 73 female subjects to determine the effects on the elasticity, firmness, and presence of fine lines and wrinkles of the skin. A seven day conditioning period was used prior to initiation of the study, where subjects were instructed to discontinue use of all moisturizing products, sunscreens and liquid make-ups, and to avoid excessive UV exposure and tanning salons. Subjects were permitted to use their current eye, powder blush, and lip products, and non-moisturizing soap.

Test subjects not in the control group, which consumed placebo tablets, consumed two (2) tablets of the test material of Example 3 daily with meals. Before, and after two (2) and five (5) weeks of tablet use, the subjects were measured as described below. Before measurements were taken, all subjects were allowed to equilibrate for thirty minutes at approximately 68° F. and 44 percent relative humidity. At each measurement phase, three Corneometer readings, a negative impression using Silflo replicating material, and three Ballistometer and Cutometer readings were made on the test sites indicated below.

A total of 65 subjects completed the study, as 7 discontinued the study for unrelated reasons and 1 developed a rash for 5 days. There were 12 subjects in the control group and 53 using the tablets.

Example 4: Image Analysis

The texture of the skin, fine lines, and wrinkles were assessed by taking Silflo replicas of the periorbital area (crow's feet) at each of the three test times. These negative impressions, or Silflo replicas, were illuminated at a precisely defined angle of 350 to create shadows for analysis by shades of gray. The skin topography is defined by the: (a)

Example 4 indicates that use of tablets prepared by the formulation of the invention herein result in a 10 percent decrease in appearance of wrinkles and an 8 percent decrease in fine lines after only 2 weeks of treatment, and a decrease of 34 percent in both wrinkles and fine lines after 5 weeks. Additionally, the observed degree of improvement is a function of the length of treatment as indicated above. This strongly suggests the treatment has imparted an improved skin infrastructure by beneficially affecting the dermis of the skin.

Example 5: Ballistometer

The Ballistometer is an instrument designed to evaluate in vivo, in a non-invasive manner, the viscoelastic properties of the skin. It analyzes the bounce pattern displayed by a probe that is allowed to impact on the skin. The kinetic energy of the probe striking the skin is stored by the elastic components of the skin and released back to make the probe rebound to a lower height. The height to which the probe will rebound depends upon the amount of stored energy lost in shear viscosity within the skin.

The capacity of the skin to absorb mechanical energy may thus be measured. Although it is unclear exactly which layer, or layers, of the skin are responsible, the mechanical properties of the dermis/epidermis layers are controlled by the density and geometry of the network of collagen fibers. It is believed the Ballistometer describes mostly the tissues underlying the stratum corneum.

Tests were conducted with the Ballistometer on one randomly chosen side of the face, slightly below the cheek bone area. The height of first rebound and the coefficient of restitution ("COR") were measured. The COR is the ratio of the first to the second rebound. Table II, below, indicates that the COR decreases by 10 percent ($p < 0.11$) and the height of

the first rebound reduced by 18 percent ($p < 0.02$) as a result of 5 weeks use of the product. This indicates that less of the energy of the striking probe was restored, thus, a greater amount of energy was dissipated in the skin. This suggests the skin became softer and more yielding during the test period.

TABLE II

	Ballistometer Readings							
	Height of First Rebound (mm)				Coefficient of Restitution			
	Mid-Baseline		Final-Baseline		Mid-Baseline		Final-Baseline	
	Control	Treated	Control	Treated	Control	Treated	Control	Treated
Average	-0.16	-0.06	0.49	0.06	-0.02	0.00	0.01	0.00
Standard Deviation	0.41	0.48	0.52	0.51	0.03	0.02	0.03	0.03
p value	$p < 0.56$		$p < 0.02$		$p < 0.06$		$p < 0.11$	
% Difference from Baseline	-6%	0%	22%	4%	-12%	-0%	12%	2%
Total % Difference (T - C)	6%		-18%		12%		-10%	

T = Treated
C = Control

Example 6: Cutometer

The Cutometer is a commercially available instrument (Courage & Khazaka, Germany) designed to measure the mechanical properties of the skin in a non-invasive manner. It measures the vertical deformation of the skin's surface when pulled by vacuum suction (500 mm Hg) through the small aperture (2 mm) of a probe and the depth of penetration of the skin into the probe optically with an accuracy of 0.01 mm. The probe is attached to a computer, which completely controls probe operation and plots skin deformation as a function of time. From this curve, a number of variables can be extrapolated to estimate the elastic, viscoelastic, and purely viscous behavior of the skin.

The following parameters were recorded: (a) the immediate distension (U_e), measured at 0.1 seconds; (b) the delayed distension (U_v); (c) the final distension (U_f), mea-

sured at 10 seconds; and (d) immediate retraction (U_r). The deformation parameters are extrinsic parameters dependent on skin thickness, and a variety of biologically important ratios were calculated: (a) U_r/U_e , a measure of net elasticity of the skin; (b) U_r/U_c , the biological elasticity, or measurement of the ability of the skin to regain its initial configu-

ration after deformation; and (c) U_v/U_c , the viscoelastic to elastic ratio, where an increase in this ratio indicates and increase in the viscoelastic portion of the deformation and/or a relative decrease of the elastic portion.

Tests were conducted using a Cutometer on both sides of the face on the cheek area. Table III, below, indicates that the delayed distension (U_v) decreased a significant 16 percent ($p < 0.04$) after 5 weeks of treatment. This parameter reflects viscoelastic properties of the skin and, thus, the behavior of the dermis. After 5 weeks, there were no statistically significant changes in U_e , the immediate distension, which is primarily affected by the moisture content and mechanical properties of the stratum corneum.

TABLE III

	Cutometer Readings							
	Mid-Baseline		Final-Baseline		Mid-Baseline		Final-Baseline	
	Control	Treated	Control	Treated	Control	Treated	Control	Treated
	U _f (mm)				U _o (mm)			
Average	0.071	0.040	0.026	0.020	0.046	0.021	0.008	0.009
Standard Deviation	0.038	0.058	0.058	0.049	0.028	0.042	0.048	0.043
p value	p < 0.11		p < 0.71		p < 0.08		p < 0.96	
% Difference from Baseline	39%	20%	16%	11%	36%	16%	11%	10%
Total % Difference (T – C)	–19%		–5%		–20%		–1%	
	U _v (mm)				U _r (mm)			
Average	0.026	0.020	0.018	0.010	0.033	0.017	0.013	0.008
Standard Deviation	0.015	0.018	0.015	0.011	0.018	0.027	0.030	0.023
p value	p < 0.27		p < 0.04		p < 0.09		p < 0.55	
% Difference from Baseline	51%	39%	34%	19%	48%	26%	19%	15%
Total % Difference (T – C)	–12%		–16%		–22%		–5%	
	U _r /U _o				U _v /U _o			
Average	0.004	0.034	0.042	0.027	0.017	0.063	0.092	0.048
Standard Deviation	0.105	0.064	0.062	0.064	0.073	0.078	0.132	0.073
p value	p < 0.21		p < 0.45		p < 0.08		p < 0.13	
% Difference from Baseline	2%	7%	9%	6%	8%	19%	28%	16%
Total % Difference (T – C)	5%		–3%		12%		–12%	

TABLE III-continued

	Cutometer Readings			
	Mid-Baseline		Final-Baseline	
	Control	Treated	Control	Treated
	U_e/U_r			
Average	0.024	0.014	0.012	0.003
Standard Deviation	0.034	0.040	0.036	0.037
p value	p < 0.47		p < 0.46	
% Difference from Baseline	6%	4%	3%	1%
Total % Difference (T - C)	-2%		-2%	

T = Treated
C = Control

Example 7: Corneometer

The general appearance of soft, smooth skin depends largely on the presence of an adequate amount of water in the stratum corneum. The Corneometer is a commercially available instrument (Courage & Khazaka, Germany) to measure the changes in capacitance of the skin resulting from changes in the degree of hydration. It is particularly sensitive to low levels of hydration, and uses measurements of arbitrary units of skin hydration (H) to express capacitance.

Tests were conducted using a Corneometer on both sides of the face on the cheek area. Changes in moisture content of the stratum corneum occur rapidly due to changes in the environment, including hydration from the use of moisturizing agents and humectants. Thus, the measurements with the Ballistometer and Cutometer indicate changes occurred in deeper layers of the skin, rather than the superficial stratum corneum. Table IV shows no significant changes in the hydration of the stratum corneum following 2 weeks (p<0.84) and 5 weeks (p<0.67) of product use.

TABLE IV

	Corneometer Readings			
	Skin Hydration (H)			
	Mid-Baseline		Final-Baseline	
	Control	Treated	Control	Treated
Average	-5	-7	-8	-4
Standard Deviation	6	7	5	7
p value	p < 0.84		p < 0.67	
% Difference from Baseline	-7%	-10%	-12%	-6%
Total % Difference (T-C)	-3%		6%	

T = Treated
C = Control

Various modifications of the invention in addition to those shown and described herein will be apparent to those skilled in the art from the foregoing description. Such modifications are also intended to fall within the scope of the appended claims. The foregoing disclosure includes all the information deemed essential to enable those skilled in the art to practice the claimed invention.

What is claimed is:

1. An orally administered pharmaceutical composition for the prevention and treatment of skin conditions in a patient comprising the following components:

a sugar compound that is converted to a glycosaminoglycan in the patient in an amount sufficient to thicken the skin;

a primary antioxidant component in an amount sufficient to substantially inhibit the activity of collagenase and elastase;

at least one amino acid component in an amount sufficient to assist in the thickening of the skin;

at least one transition metal component in an amount effective to bind collagen and elastic fibers and thicken skin; and a catechin-based component present in an amount sufficient to inhibit the presence of anti-collagen enzyme in the skin.

2. The pharmaceutical composition of claim 1, wherein the sugar compound is present in about 5 to 50 weight percent, the primary antioxidant component is present in about 5 to 50 weight percent, the amino acid component is present in about 8 to 60 weight percent, and the transition metal component is present in about 0.5 to 15 weight percent of the composition.

3. The pharmaceutical composition of claim 1, wherein the sugar compound comprises an N-acetylglucosamine compound or salt or ester thereof, the primary antioxidant component comprises ascorbic acid compound or salt or ester thereof, at least two amino acids selected from the group consisting of proline, lysine, cysteine, and methionine are present, and at least two the transition metal components comprising zinc, manganese or copper, or mixtures thereof, are present.

4. The pharmaceutical composition of claim 3, wherein at least three transition metal components are present, one of which is zinc monomethionine, one of which is manganese ascorbate, and one of which is copper sebacate, wherein the zinc is present in about 10 to 30 weight percent of the complex and the manganese is present in about 5 to 20 weight percent of the complex, and the copper is present in about 5 to 20 weight percent of the complex.

5. The pharmaceutical composition of claim 3, wherein the N-acetylglucosamine is present in about 5 to 30 weight percent, the ascorbic acid is present in about 5 to 50 weight percent, the amino acid component comprises lysine and proline, wherein each is present in about 4 to 25 weight percent, and the zinc monomethionine and the manganese ascorbate are each present in about 1 to 10 weight percent and the copper sebacate is present in about 0.1 to 5 weight percent of the composition.

6. The pharmaceutical composition of claim 1, wherein the composition further comprises a pharmaceutically acceptable carrier or excipient.

7. The pharmaceutical composition of claim 1, further comprising a catechin-based preparation, a glucosamine or a pharmaceutically acceptable salt or ester thereof, and a chondroitin or a pharmaceutically acceptable salt or ester thereof.

8. The pharmaceutical composition of claim 7, wherein the catechin-based preparation is a proanthanol or proanthocyanidin, and the glucosamine and chondroitin are each a sulfate or succinate.

9. The pharmaceutical composition of claim 8, wherein the proanthocyanidin is grape seed extract present in about 0.5 to 5 weight percent, the glucosamine is D-glucosamine sulfate present in about 3 to 17 weight percent, wherein the glucosamine is about 60 to 90 weight percent of the salt, and the chondroitin is chondroitin sulfate present in about 3 to 17 weight percent of the composition, wherein the chondroitin is preferably present as about 65 to 95 weight percent of the salt.

10. The pharmaceutical composition of claim 7, further comprising a vitamin E source, a cysteine source, a vitamin B₃ source, quercetin dihydrate, pyridoxal 5 phosphate-Co B₆, a methionine source, and a vitamin A source.

11. The pharmaceutical composition of claim 10, wherein the vitamin E is D-alpha tocopheryl acid succinate present in about 1 to 15 weight percent, the vitamin B₃ is niacinamide present in about 0.5 to 15 weight percent, the vitamin A is vitamin A palmitate present in about 0.1 to 5 weight percent, the cysteine is N-acetyl cysteine present in about 1 to 10 weight percent, the methionine is preferably L-selenomethionine present in about 0.1 to 5 weight percent, the quercetin dihydrate is present in about 0.5 to 15 weight percent, and the pyridoxal 5 phosphate-Co B₆ is present in about 0.1 to 5 weight percent of the composition.

12. An orally administered pharmaceutical composition for the prevention and treatment of skin conditions in a patient comprising:

an N-acetylglucosamine compound, or a pharmaceutically acceptable salt or ester thereof, present in about 5 to 30 weight percent;

an ascorbic acid compound, or a pharmaceutically acceptable salt or ester thereof, present in about 5 to 50 weight percent;

at least two different amino acid compounds wherein at least one amino acid compound is proline, lysine, cysteine, or methionine and each amino acid is present in about 4 to 25 weight percent; and

at least one transition metal component wherein at least one transition metal compound is zinc, manganese, or

copper, or mixtures thereof, present in about 0.5 to 15 weight percent to thicken skin.

13. A method for the prevention or treatment of skin conditions, wherein the skin has a thickness of dermis and collagen, which comprises orally administering to a patient a pharmaceutical composition comprising:

a sugar compound that is converted to a glycosaminoglycan in the patient in an amount sufficient to thicken the skin;

a primary antioxidant component in an amount sufficient to substantially inhibit the activity of collagenase and elastase;

at least one amino acid component in an amount sufficient to assist in the thickening of the skin; and

at least one transition metal component in an amount effective to bind collagen and elastic fibers and thicken skin, said composition administered in an amount therapeutically effective to modify the thickness of the skin to prevent or treat at least one skin condition.

14. The method of claim 13, wherein the skin condition prevented or treated is at least one of wrinkles or the appearance thereof, fine lines or the appearance thereof, thinning, reduced skin elasticity, reduced skin moisture, spider veins, senile purpura, sun damaged skin, aging skin or rough skin.

15. The method of claim 12, wherein the composition is administered as a tablet or capsule having about 1 mg to 2,000 mg of composition.

16. The method of claim 14, wherein the tablet or capsule has about 200 mg to 1,600 mg of composition.

17. The method of claim 15, wherein the tablet or capsule has about 600 mg to 1,000 mg of composition.

18. The method of claim 13, wherein the composition is administered in conjunction with concurrent or subsequent treatment by at least one additional pharmaceutical composition for the prevention or treatment of a skin condition.

19. The method of claim 13, further comprising providing a catechin-based component present in an amount sufficient to inhibit the presence of an anti-collagen enzyme in the skin.

* * * * *

EXHIBIT C

Ascorbic Acid: Chemistry, Metabolism, and Uses

Paul A. Seib, EDITOR
Kansas State University

Bert M. Tolbert, EDITOR
University of Colorado

Based on a symposium
sponsored by the Division of
Carbohydrate Chemistry
at the Second Chemical Congress
of the North American Continent
(180th ACS National Meeting),
Las Vegas, Nevada,
August 26-27, 1980.

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7	43.8
3	75.0
4	33.3
41	80.4
1	14.3
3	60.0
11	78.6

36. Copyright 1969, Veterinary

a period of years. Other
for which there is a lack
with high levels of ascorbic

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nsidered for a short period
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administration is the most
of need. For longer term
suming adequate stability

e of feed to high humidity,
ied ascorbic acid. Pelleting
hot water and subsequent
id. Attempts to overcome

this destructive action include the use of coated ascorbic acid product forms and the applicati n f ascorbic acid to the pellet feed following the pelletizing process. L-Ascorbic acid coated with ethyl cellulose (4%) and finely powdered ascorbic acid uniformly blended into a warm edible hydrogenated fat (50%) and immediately sprayed in chilled air into small beadlet form are two methods that have improved stability in animal feeds (Table VIII). Even with these products, an excess over claim values must be added.

If solid fat is an ingredient of the feed as an energy source, one practical way of adding ascorbic acid is to uniformly suspend the finely powdered product into the liquified hydrogenated fat and spray the suspension onto the cooled pelleted feed in a tumbler or on a moving belt conveyor. Andrews and Davis (239) reported this method to be feasible for pelleted fish feed. Heat-expanded commercial catfish feed (32% protein) was sprayed with a warm fat suspension of ascorbic acid to 2, 5, and 10% fat coating on the feed. After drying, the water stability (water leaching of ascorbic acid) of the product was evaluated (Table IX) as fish feed is normally cast on water for fish consumption.

A problem arose as how to add L-ascorbic acid to baked monkey biscuits since baking is quite destructive to ascorbic acid. After some trial work it was discovered that ascorbic acid (10–20%) in concentrated

Table VIII. Ascorbic Acid Stability in Unpelleted Feed

Type Product	Storage Condition	
	3 Weeks/45°C 100% Humidity	6 Weeks/45°C Room Humidity
	(% Retention Ascorbic Acid)	
Ascorbic acid, crystalline	0	40–80
Ethocel coated, AA	0	84–87
Fat (50%) beadlets, AA	78–84	91–99

Table IX. Retention of Ascorbic Acid* in Fabricated Fish Feeds

Ascorbic Acid Level mg/kg Diet	Ascorbic Acid (%) Remaining in Feed Particles After Water Exposure		
	1 Minute	5 Minutes	10 Minutes
90	96	74	50
197	86	67	42
379	83	67	36

* Ascorbic acid in a warm fat suspension sprayed on feed after pelleting.
Source: Reproduced, with permission, from Ref. 239. Copyright 1979, Miller Publishing Company.

sucrose syrup (50-60%) could be brushed, sprayed, or dropped on to the biscuits after baking with fairly good stability performance. Ascorbic acid is quite stable in high sugar composition such as candy, added at the last stage of processing because of the dense nature of the high sucrose products and relative freedom from oxygen.

Miscellaneous. In miscellaneous animal applications relating to the use of ascorbic acid, interactions with toxic levels of minerals have been observed. Hill (240) found increased dietary ascorbic acid in chickens was specific in counteracting metal toxicity in cases of selenium and vanadium. This was also noted by Berg et al. (241). Cadmium toxicity in the rat (242) and in Japanese quail (243, 244) has been reversed or counteracted. Ascorbic acid (500 mg/kg) given intraperitoneally before or simultaneously with an oral dose of paraquat altered the activity of paraquat indicating ascorbic acid to be an effective detoxifying agent (245). Kallistratos and Fasske (246) noted inhibition of benzo[a]pyrene carcinogenesis in rats with ascorbic acid treatment. There is a decreased level of ascorbic acid in lung tissue of mice following exposure to ozone, and Kratzing and Willis (247) propose that one function of tissue ascorbic acid may be an extracellular antioxidant in the lungs. Use of ascorbic acid and mineral supplements in the detoxification of narcotic addicts (248, 920) has been discussed. While not a practical approach, the injection of young chickens with sodium ascorbate has been reported to heal bruises at a faster rate than in nontreated animals (249).

Pharmaceutical Applications

L-Ascorbic acid (vitamin C) is an active ingredient in a variety of pharmaceutical dosage forms such as: high-potency multivitamin supplement; high-potency multivitamin supplement with iron; high-potency multivitamin supplement with minerals; vitamin B complex; vitamin B complex with vitamin E; pediatric drops; tablets of a range of potencies; injectables; and syrups and elixirs. An alternative list of pharmaceutical dosage forms containing ascorbic acid would be: tablets with a wide range of potencies, drops (especially for pediatric use), injectables, syrups and elixirs, effervescent tablets, and multivitamin preparations.

Many vitamins are quite stable under normal processing conditions and present little or no stability problems in finished pharmaceutical products. These include biotin, niacin, niacinamide, pyridoxine, riboflavin, and α -tocopheryl acetate. Others that can present problems are ascorbic acid, calciferol, calcium pantothenate, cyanocobalamin, folic acid, and retinyl esters. Overages above label claim are customarily added to vitamin formulations as a means of maintaining the claimed level of each vitamin for the expected shelf life of the products. The percent overage for a particular vitamin such as L-ascorbic acid will vary

sprayed, or dropped on to lity performance. Ascorbic n such as candy, added at dense nature of the high oxygen.

applications relating to the vels of minerals have been y ascorbic acid in chickens in cases of selenium and (241). Cadmium toxicity (244) has been reversed or en intraperitoneally before quat altered the activity of effectiv detoxifying agent hhibition of benzo[a]pyrene ment. There is a decreased ollowing exposure to ozone, ne function of tissue ascor- in the lungs. Use of ascor- ification of narcotic addicts a practical approach, the rbate has been reported to d animals (249).

e ingredient in a variety of tency multivitamin supple- at with iron; high-potency min B complex; vitamin B lets of a range of potencies; ative list of pharmaceutical d be: tablets with a wide tric use), injectables, syrups amin preparations.

normal processing condi- blems in finished pharma- n, niacinamide, pyridoxine, that can present problems nate, cyanocobalamin, fola- bel claim are customarily of maintaining the claimed life of the products. The as L-ascorbic acid will vary

according to its performance pattern. In general, problems of instability of vitamins are much more acute in multivitamin liquids than in single vitamin formulations or in solid dosage forms.

Solid Dosage Forms. L-Ascorbic acid tablets constitute one of the major uses in pharmaceutical applications. Tablets may be of the coated or uncoated type, in various potencies and sizes, and also swallowable or chewable. These solid dosage forms are prepared either by double compression or slugging, by wet granulation, or by direct compression. In the usual process of tablet preparation ascorbic acid in powder or fine granular form together with suitable diluents of lactose, sucrose, or starch with lubricant is slugged and reduced to granules, then recompressed into tablets of the desired size. An alternate method consists of making a moist paste of lactose, starch, and sucrose, which is screened, dried, and reduced to granules, then mixed with ascorbic acid in coars crystalline form and lubricant and compressed into tablets. Special L-ascorbic acid application forms are available that permit direct compression into tablet form. Chewable tablets contain sodium ascorbate in addition to ascorbic acid and flavoring agents to provide a more pleasant taste. Another special type of solid dosage form is the effervescent tablet, usually of higher potency (0.5–2.0 g) that is consumed when added water converts the tablet to a liquid preparation. There are a number of scientific papers and patents detailing the formulation and manufacture (250–257) of solid ascorbic acid dosage forms, their stability (258–266), in vitro release of nutrients (267, 268, 269), and bioavailability (270–275). Data (Table X) collected on commercial ascorbic acid tablets stored at room temperature (25°C) demonstrate full label potency over a shelf life period of many years. Under normal storage conditions, commercial type ascorbic acid tablets are stable for over 5 years (> 95% potency retention). The amount of three breakdown products (dehydroascorbic acid, diketogulonic acid, and oxalic acid) formed under various storage conditions constitutes a small percentage of the ascorbic acid content and poses no dietary hazard (276). In the application of sugar coating to multivitamin tablets, careful technique is required to prevent excessive penetration of moisture into the tablet core, which can lead to high losses of vitamins sensitive to moisture and pH influences.

Liquid Dosage Forms. In dry form and at very low moisture content, L-ascorbic acid is very stable, but in solution exposed to air or oxygen it is subject to oxidation accelerated by dissolved trace minerals (copper and iron) and light exposure. L-Ascorbic acid is a reducing agent and is subject to oxidative decomposition in solution. This proceeds first to dehydroascorbic acid, which has full vitamin C activity, but continues to diketogulonic acid and various other breakdown products. The degradation reactions are complex and vary with aerobic or anaerobic

Table X. Stability of
Analysis of Ascorbic Acid, 100-mg Tablets,
After Long-Term Storage at 25°C

Lot Number	Storage Time (months)	Assay (% of claim)*	Initial Assay (% of claim)*
V-418	103	99	103
KRK-202-66I	103	104	107
KRK-202-65-III	103	106	110
KRK-202-65-IV	103	106	110
KRK-202-66-II	103	105	111
DMS-289-II	90	104	102
Lot 2082	120	111	—
Lot 2964B	240	101	—
Lot 002-0B097A	96	98	—

* Assay by iodometric and 2,6-dichloroindophenol titrations.

situations, the nature of the formulation, and the type of stress to which pharmaceutical solutions are subjected. Ascorbic acid degradation is also pH dependent. Under aerobic conditions, the rate of oxidation shows maxima at pH 5, corresponding to reaction with 1 equivalent of base, and at pH 11.5, corresponding to reaction with 2 equivalents of base. A pH-log rate profile (pH range of 3.5–7.2) for rate of aerobic oxidation of aqueous ascorbic acid solutions (67°C) was determined by Blaug and Hajratwala (277). A first-order degradation was observed. Rogers and Yacomini (278) also studied the effect of pH on ascorbic acid solutions (25°C).

Under anaerobic conditions, the dependency of the stability of ascorbic acid in aqueous solutions on pH is relatively low, but there is a maximum rate of degradation, which is equal to the pK_{a1} of ascorbic acid, at a pH of about 4.1. Stability of ascorbic acid in multivitamin drops has been studied at various pH levels. Maximum losses occur in the pH range of 3.3 to 4.5 and smaller losses are found at higher pH (up to 5.5). Figure 6 shows stability data for ascorbic acid in multivitamin elixir preparations for teaspoon dosage for storage at 45°C. The pH of such solutions has a more pronounced effect than in multivitamin drops. Losses at pH 3.5 are as high as 40% in 6 weeks at 45°C. Rate studies on the anaerobic degradation of ascorbic acid as influenced by metal ions. Finholt et al. (279, 280), have indicated the involvement of salt-acid and metal complex formations.

During the past two decades ascorbic acid free radicals have become recognized and their kinetics studied (281–287) in the oxidation of ascorbic acid. Interactions between certain of the vitamins or ingredients

Table X. Stability of

Ascorbic Acid, 100-mg Tablets,
1-Term Storage at 25°C

Assay (% of claim)*	Initial Assay (% of claim)*
99	103
104	107
106	110
106	110
105	111
104	102
111	—
101	—
98	—

mol titrations.

and the type of stress to which ascorbic acid degradation is also, the rate of oxidation shows an inverse relationship with 1 equivalent of base, and with 2 equivalents of base. The rate of aerobic oxidation was determined by Blaug and Rogers. The effect of pH on ascorbic acid

dependency of the stability of ascorbic acid is relatively low, but there is a relationship equal to the pK_{a1} of ascorbic acid in multivitamin preparations. Maximum losses occur in multivitamins at higher pH for ascorbic acid in multivitamins for storage at 45°C. The pH effect is more pronounced in multivitamin preparations than in ascorbic acid as influenced by storage. This has indicated the involvement of

ascorbic acid free radicals have become involved in the oxidation of the vitamins or ingredients

Vitamin C Tablets

Analysis of Ascorbic Acid, 100-mg Tablets,
After Long-Term Storage at 25°C

Loss in Potency (%)	Diketogulonic Acid (%)	Oxalic Acid (%)	Dehydroascorbic Acid (%)
4	1.0	0.5	0.5
3	2.0	0.5	0.2
4	1.0	0.5	0.2
4	1.0	0.5	0.4
6	2.0	1.0	2.4
—	0.5	0.5	0.3
—	—	0.2	0.4
—	—	0.4	2.3
—	—	—	1.3

Source: Reproduced, with permission, from Ref. 276. Copyright 1976, American Pharmaceutical Association.

are of great interest to the pharmaceutical chemist both from the theoretical and the practical viewpoints.

1. Hand, Guthrie, and Sharp (288) reported that riboflavin catalyzes the photochemical oxidation of ascorbic acid that occurs in the presence of oxygen on exposure to light. Conversely, ascorbic acid exerts a reducing effect on riboflavin, which is very likely involved in the formation of chloroflavin in B-complex solutions containing ascorbic acid.
2. A yellow complex of 1 molecule of niacinamide with 1 molecule of ascorbic acid also forms readily in solution by what appears to be a charge-transfer reaction. The complex has been prepared in solid form. It has been claimed that the preforming of this complex presents difficulties with thickening and hardening of mixtures employed in soft gelatin capsules. Guttman and Brooke (289) found the extent of association between niacinamide and ascorbic acid to be pH dependent with maximum adsorbance at pH 3.8.
3. In acid medium the folic acid molecule is cleaved by reducing agents such as ascorbic acid. This reaction occurs in two stages: (i) cleavage to the pteridine moiety plus p-aminobenzoylglutamic acid, and (ii) destruction of the free amino group of the p-aminobenzoylglutamic acid. The decomposition of folic acid is more rapid at pH 3 than at pH 6.5.
4. Stabilization of vitamin B₁₂ solutions in the presence of thiamine, niacinamide, and ascorbic acid has been the subject of a number of patents. Newmark (290) has described

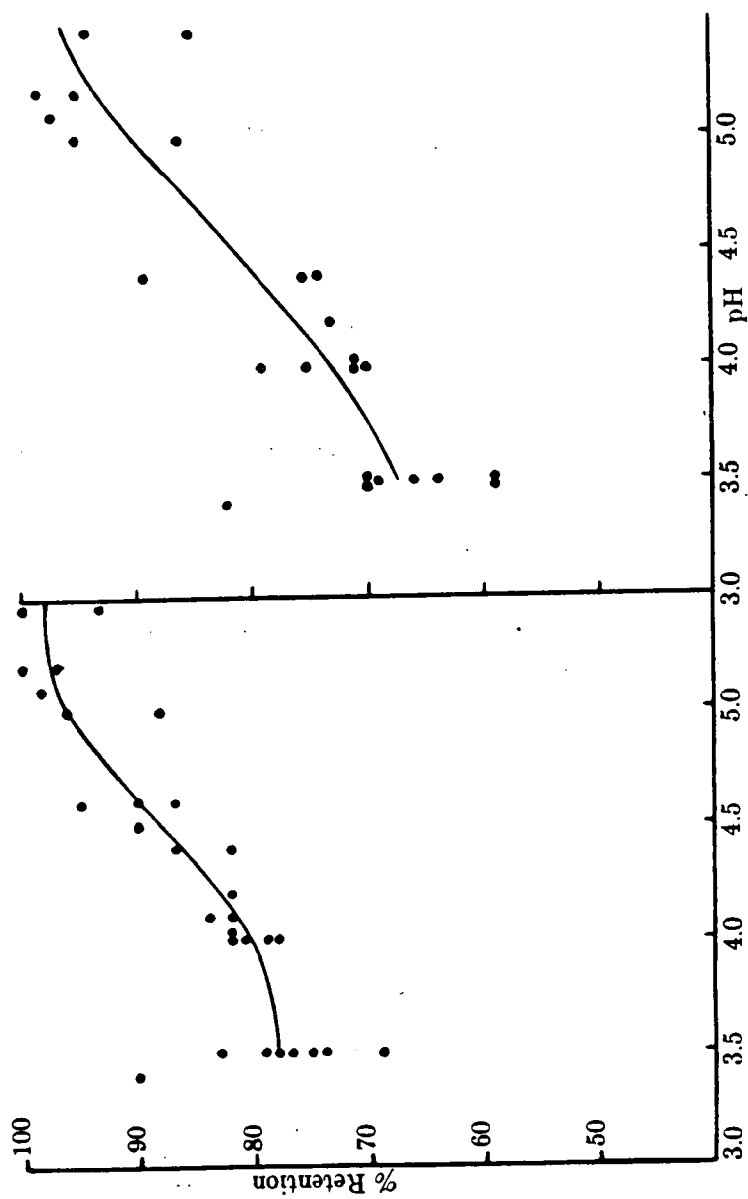


Figure 6. Ascorbic acid in multivitamin preparations for teaspoon dosage. Left, 3 weeks/45°C (5 products); right, 6 weeks/45°C (4 products).

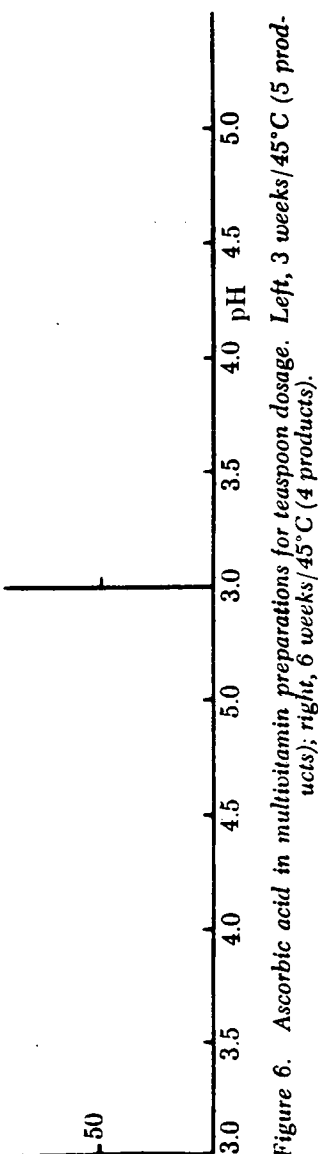


Figure 6. Ascorbic acid in multivitamin preparations for teaspoon dosage. Left, 3 weeks/45°C (5 products); right, 6 weeks/45°C (4 products).

the effective stabilization of B₁₂ in such solutions by various iron compounds and salts.

5. Ascorbic acid will destroy many of the FD&C azo colors in solution, so stable colorants must be chosen.
6. The decomposition of ascorbic acid is catalyzed by trace metal ions in solution, hence the addition of sequestering agents such as EDTA and its salts has been shown to enhance the stability of ascorbic acid (291, 292, 293).

A review of such interactions was published by Scheindlin (294) in 1958. Porikh and Lofgren (295) demonstrated increased stability of ascorbic acid, confirmed by Kato (296), when glycerin and/or propylene glycol type products were substituted for part of the water in an oral multivitamin liquid. Baudelin and Tuschhoff (297) reported similar findings on ascorbic acid and, in addition, found that ethanol or sugars such as sucrose, corn syrup, and dextrose also provide a stabilizing effect on ascorbic acid. Paust and Coliazzi (298) and Fabrizi et al. (299) found that the first-order rate constants for oxidative decomposition of ascorbic acid decrease as a function of polysorbate 80 concentration up to 30% at 30°C. Similar findings were observed by Nixon and Chawla (300) with polysorbate 20. Other reports (301–309) deal with factors influencing formulation and stability of liquid dosage forms of ascorbic acid.

Sterile aqueous solutions prepared with high purity ascorbic acid and pyrogen-free distilled water in glass-lined equipment under absolute sanitary operations and filled into ampules are necessary for injectable solutions for parenteral use in humans and animals. For all injectable products, it is important to select container, stopper, preservative, and other ingredients that are compatible.

The formulator of liquid multivitamin pharmaceutical products such as baby drops, syrups, elixirs, and injectables encounters numerous problems in attempting to develop products having adequate physical and chemical stability as well as suitable taste, odor, color, and freedom from bacterial contamination. Many of these problems arise from the differing solubility and stability characteristics of the individual vitamins, particularly as these relate to the pH of the solutions and potential interactions. Despite these numerous problems, various ways have been devised for producing multivitamin combinations in liquid form containing L-ascorbic acid that have acceptable stability characteristics. Successful development of such products requires a knowledge of: (i) the fundamental aspects of the physical and chemical properties of the vitamin forms available; (ii) the use of adequate techniques of manufacture; and (iii) the employment of suitable overages based on critical stability studies.

Food Applications

L-Ascorbic acid may be added to foods or food ingredients as a nutrient to fortify natural foods having little or no vitamin C, to restore losses, to standardize a given class of food products to having a pre-selected quantity, and to endow or enrich synthetic foods with nutritional value. The term nutrification is used to cover all the above situations for adding a nutrient to a food product. To nutrify a food or to make it a nutrified food implies an act to make the food more nutritious. Adding micronutrients such as vitamins, minerals, amino acids, and vitamin A active carotenoid colorants to a food at low cost for nutritional improvement is not a new concept. Iodine was added to salt in the 19th century in South America. Nutrification is a most rapid, most economical, most flexible, and most socially acceptable method of changing the nutrient intake of a given population (310).

New technological advances enable the food processing industry to market many more food products than decades past. Factors that must be considered in conjunction with appropriate technology before added ascorbic acid is considered are: cost of the specific food; convenience of use; relationship of the nutrient to the usual food selection pattern or other replacement or supplemental food products; stability of the nutrient in the food during market shelf life and home preparation; special food needs, for example, infant, geriatric, or military; public health considerations.

L-Ascorbic acid is also added to food in essentially a non-nutrient capacity such as a preservative or oxygen acceptor, as an acidulant, as a stabilizer of cured meat color, or as a flour improver. Because of the ene-diol group, it has a marked inhibitory influence on the oxidation-reduction reactions responsible for undesirable color, flavor, and odor development. Its mechanism of action is dependent upon the characteristics of the food or food ingredient, the associated environments, the processing technology, and the storage expectancy of the product.

The food processing industry can obtain L-ascorbic acid and sodium ascorbate commercially in a variety of mesh sizes to meet the requirements of various kinds of food products. These crystalline compounds are stable for years when stored under cool, dry conditions in closed containers. Esters of ascorbic acid such as ascorbyl palmitate are also available.

Addition Methods. The four basic technologies developed for adding ascorbic acid to foods are:

1. *Tablets or wafers.* Compressed soluble discs containing inert, edible carriers and sufficient ascorbic acid to meet the ascorbic acid regulatory and (or) processing requirements of a given quantity of food. The tablet added to

ods or food ingredients as a
 ble or no vitamin C, to restore
 od products to having a pre-
 ynthetic foods with nutritional
 cover all the above situations
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 nt ascorbic acid to meet
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 od. The tablet added to

the container prior to filling and sealing of liquid foods
 dissolves immediately, or may be dissolved and added to
 semisolid foods or dry foods at a late stage of food
 preparation.

2. *Dry premixes.* A uniform mixture of a known amount of ascorbic acid and a dry carrier, usually a constituent of the food. The premix blended with a prescribed quantity of dry food product gives a greater assurance of product uniformity since the quantity of the pure vitamin may be small.
3. *Liquid sprays.* Sprays of ascorbic acid solutions or suspensions that may be considered liquid premixes. The sprays are directed onto the surface of a food or injected into liquid food products to circumvent difficult or continuous processing conditions. For example, toasted ready-to-eat cereals are fortified by spraying a solution onto flakes still warm from the toasting process.
4. *Pure compound.* Crystalline ascorbic acid, sodium ascorbate or special coated product forms are widely added directly to food or predetermined quantities often in the form of preweighed packets for convenience. Addition is accompanied by mixing to ensure uniformity.

Hundreds, if not thousands, of reports have been published on the application of ascorbic acid to food products for either nutritional objectives or improvement in food quality. Bauernfeind reviewed the use of ascorbic acid (311, 312) in processing food in 1953 (406 references) and again in 1970 (520 references); other reviews (313-332) on food applications of ascorbic acid have appeared prior to and following these dates.

The stability of ascorbic acid is influenced by atmospheric oxygen, water activity, oxidative enzymes, pasteurization methods, metal contamination, and sulfur dioxide content.

The degradation of ascorbic acid in foods has been widely studied. It is complex in nature and depends on specific conditions and the presence of other substances. While degradation is not a topic of this review a few food related references are included as an introduction to the literature on this subject. Ascorbic acid has the ability to scavenge superoxide and hydroxyl radicals as well as singlet oxygen (333). In a 1975 report on the destruction of ascorbic acid as a function of water activity by Lee and Labuza (334), the half-life of ascorbic acid (Figure 7) is well illustrated as a function of moisture content. Degradation compounds formed by heating ascorbic acid in solution were identified by Tatum et al. (335) and Kamiya (336). Thompson and Fennema (337) observed differences in the effect of freezing on the rate of oxidation of ascorbic acid in foods as compared with dilute simple solutions. Timberlake (338) found the oxidation of ascorbic acid in the presence of metals was significant and could be influenced by metal chelating

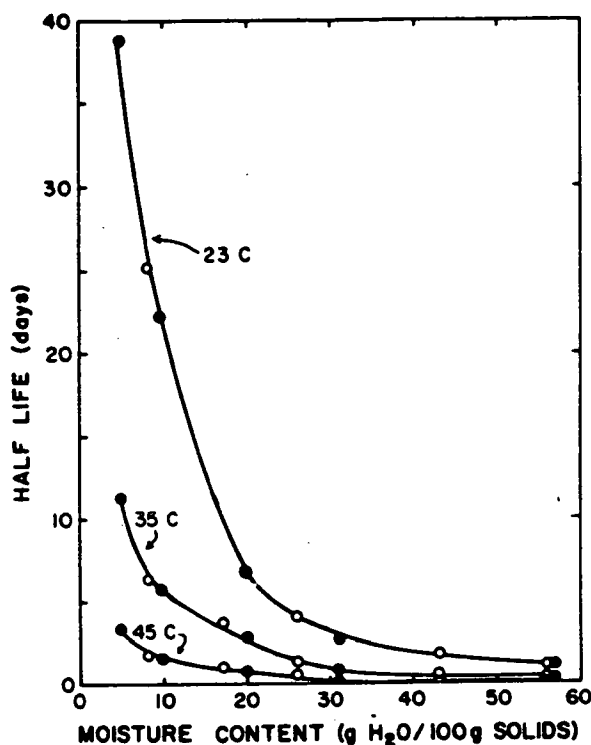
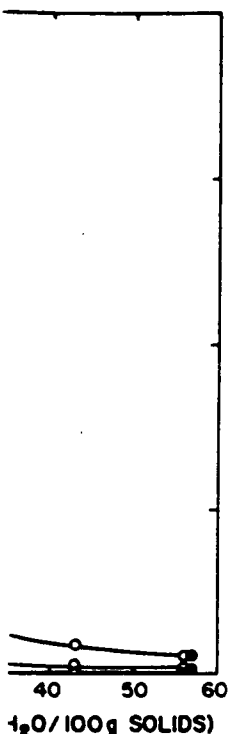


Figure 7. Half-life for ascorbic acid as a function of moisture content. Key: ●, DM; ○, DH. (Reproduced, with permission, from Ref. 334. Copyright 1975, Institute of Food Technologists.)

agents in black currant juice. In the last decade more attention has been given to kinetics of quality degradation including ascorbic acid oxidation in food products or model systems. Examples are moisture-sensitive products (339), ascorbic acid oxidation in infant formula during storage (340), ascorbic acid stability of tomato juice as functions of temperature, pH, and metal catalyst (341), the degradation of ascorbic acid in a dehydrated food system (342), and the oxygen effect on the degradation of ascorbic acid in a dehydrated food system (343).

The point in the food manufacturing process at which ascorbic acid is introduced is important. Ideally, it is added as close to the terminal process stage as possible, when conditions allow. To maximize the stability and efficacy of ascorbic acid added to foodstuffs, the following precautions are recommended for practical success according to Klaeui (317):

1. Direct contact of the food product or its ingredients with brass, bronze, monel, steel, and iron must be avoided.



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ct or its ingredients with
iron must be avoided.

2. The equipment used should be of stainless steel, aluminum, enamel, glass, china, or approved plastic.
3. Wherever possible, deaeration should precede processing, which should be carried out under inert gas or in a vacuum.
4. During mixing, emulsification, homogenization, and the like, oxygen or air should not be introduced into the product.
5. Where possible, food product should be protected from light and other radiant energy.
6. Containers should be filled to maximum capacity, that is, the headspace should be kept as small as possible.
7. After heat processing of sealed containers, rapid cooling should follow and the products should be stored at cool temperatures.
8. If practicable, sequestering agents such as phosphates, citrates, EDTA, or cysteine may be added.
9. All autoxidizable ingredients, such as flavoring oils, added to the food product should have a low peroxide value.
10. Whenever feasible, a short-time heat treatment of fresh food products should be employed to inactivate enzymes before adding ascorbic acid.
11. Where feasible, microorganisms may be removed by filtration or inactivated by heat treatment and, if possible, processing may be continued under aseptic conditions.
12. Preferably, some ascorbic acid should be present prior to bottling or canning (1 mL of residual air reacts, theoretically, with 3.3 mg of ascorbic acid).

L-Ascorbic acid is found in all living tissues, both animal and plant matter, and as such has been consumed by humans for thousands of years, thus giving encouragement that the compound is physiologically acceptable and safe. Furthermore, extensive testing of L-ascorbic acid prepared by chemical synthesis confirms its relative safety. Large-scale manufacture, coupled with high standards of purity and relatively low cost, makes application to food products economically feasible.

Use as a Nutrient. Processing never makes a food product more complete than the original fresh product nor can it compensate for nature's idiosyncrasies in content of original nutrients. However, the preservation, processing, and storage of food are necessary to provide palatability, safety to health, variety of selection, and provision for future use. In the production, handling, preserving, processing, and storage of food, some nutrients are lost or significantly lowered. Not only does the nutrient content vary in the natural whole-plant food—because of variety, climate, harvesting methods, storage—but processing (because of exposure to heat, oxygen, metals, and so forth, and food-fractionation processes) modifies the nutrient content. One nutrient especially sensitive to

some of the factors mentioned is L-ascorbic acid. Furthermore, not only are the traditional natural and processed or refined foods sold today, but also new "convenience," "semblance," "fabricated," "novel," and "dietetic or low-calorie" foods. Some of these products simulate known foods. Others have no past counterpart and may have low levels of micronutrients such as L-ascorbic acid, which can be corrected by nutrition. Many countries (344) have established a recommended daily allowance or intake of ascorbic acid for humans (Table XI).

FRUIT BEVERAGE PRODUCTS. L-Ascorbic acid has been associated with fruit and fruit juices since 1753 when Captain James Lind, physician to the British Fleet, demonstrated the successful treatment of scurvy by incorporating citrus juices in the diet. The pattern of fruit products consumption has undergone a gradual change over the years. The decline of whole fresh fruit and vegetable consumption is correlated with increased use of frozen or canned fruits, vegetables, and juices and, more recently, reconstituted fruit-flavored beverages. Fruit and vegetable products are the primary sources of L-ascorbic acid in this diet. Apple, grape, pineapple, prune, and cranberry juices and peach and apricot nectars contain little or no ascorbic acid unless nutrified. Other juices may be variable sources. Fruit juices low in ascorbic acid are used interchangeably with those of high ascorbic acid content. It is the

Table XI. International Dietary Allowances for Vitamin C

Country	Adult Male	Pregnancy	Lactation
Australia	30	60	60
Canada	30	40	50
Columbia	50	65	65
Finland	30	50	90
East Germany	70	100	100
West Germany	75	100	120
INCAP	55 (50) *	60	60
India	50	50	80
Indonesia	60	90	90
Japan	60 (50) *	60	90
Malaysia	30	60	60
Netherlands	50	75	75
Philippines	75 (70) *	100	150
Thailand	30	50	50
Turkey	50	70	80
United Kingdom	30	60	60
United States ^b	60	80	100

Note: Values are given in milligrams per day.

* Female.

^b NRC-1980 allowance.

Source. Reproduced, with permission, from Ref. 344. Copyright 1975, Common Agricultural Bureaux.

acid. Furthermore, not only refined foods sold today, "fabricated," "novel," and these products simulate known and may have low levels of which can be corrected by nutrition. A recommended daily allowance (Table XI).

Ascorbic acid has been associated with Captain James Lind, physician to the successful treatment of scurvy by the pattern of fruit products change over the years. The daily consumption is correlated with fruits, vegetables, and juices and beverages. Fruit and vegetable L-ascorbic acid in this diet. Berry juices and peach and ascorbic acid unless nutrified. Other products low in ascorbic acid are ascorbic acid content. It is the

contention of some groups that it is desirable to nutrify those juices to make them more comparable with the latter from a standpoint of nutritive value, hence, leaving choice of selection based on flavor preferences.

Ascorbic acid levels of 30–60 mg/100–200 mL are meaningful concentrations. For example, Del Monte (345) began adding L-ascorbic acid to tomato juice (60 mg/180 mL) on a commercial basis in 1974. This conforms to U.S. FDA regulations. The effect of processing variables and product storage in ascorbic nutrified tomato juice has been studied by Flinn (346) and by Pope and Gould (347). In addition to the straight fruit juices, different beverage-based products have been studied as ascorbic acid nutrified foods; such products include a chocolate-flavored powder (348), a whey-soy drink mix (349), malt lemonades (350), and fruit juice carbonated beverages (351, 352). L-Ascorbic acid stability, its influence on quality during storage, and its influence on processing fruit juices and beverages remain a subject of active study (353–359).

The acid-type fruit and vegetable juices are good carriers of ascorbic acid, thus providing a relatively stable environment. Gresswell (322) and others in the past (311, 312, 318) have reviewed the use of L-ascorbic acid in beverages and fruit juices. In adding ascorbic acid to juices and beverages, a decision must be made about the nutrient level to be claimed for the product in the market place plus that expected to be destroyed in processing and during shelf life. It must be recognized that oxidative enzymes (ascorbic acid oxidase, peroxidases) exist in fruit and need to be heat inactivated. Removal of oxygen or air by vacuum deaeration and replacement by nitrogen or carbon dioxide or flushing headspace with inert gas will reduce ascorbic acid destruction. In some instances the addition of glucose oxidase and catalase is useful in removing dissolved and headspace oxygen, which minimizes required ascorbic acid addition. Use of minimum amounts of sulfur dioxide (insufficient to cause flavor changes) can be helpful in some products for better ascorbic acid retention values.

Exposure of ascorbic acid nutrified juices and beverages to direct sunlight, depending on the liquid formulation, can accelerate destruction of ascorbic acid and bring about flavor changes. If the product will be exposed, choice of packaging may minimize light influences. Oxygen permeability of packaging material should not be overlooked. In any new product to be nutrified, pilot-size production batches (Table XII) should be run with the best selected variants and storage data obtained before commercial production is commenced. Added L-ascorbic acid in dry, fruit-flavored powdered products to be reconstituted is quite stable if moisture levels are kept low and if they are packed in laminated, moisture-resistant packets.

It has been recognized that the elderly (360) do not always consume sufficient L-ascorbic acid due, in part, to lack of selection of appropriate

Allowances for Vitamin C

Pregnancy	Lactation
60	60
40	50
65	65
50	90
100	100
100	120
60	60
50	80
90	90
60	90
60	60
75	75
100	150
50	50
70	80
60	60
80	100

EXHIBIT D

S.T.P. Pharma (4), 1985

281-284

⑧ Coll. Pharmacy

⑧ Detroit ⑨ MI ⑩ US

⑧ Wayne State University

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Vitamin C is very widely used but its instability is well-known.

The author reviews the conditions of its degradation in aerobic and anaerobic media. He then considers the influence of different factors such as pH, and of various catalysts such as metallic ions. He then discusses the processes that make it possible to improve the stability of vitamin C.

Keywords : Vitamine C - Ascorbic acid - Stability - Catalysts - Preservation.

La vitamine C est très largement utilisée mais son instabilité est bien connue.

De ce fait, l'auteur passe en revue les conditions de sa dégradation, en milieu aérobie et anaérobie. Il envisage ensuite l'influence de différents facteurs comme le pH et divers catalyseurs tels que les ions métalliques. Enfin, il aborde les procédés qui peuvent permettre d'améliorer la stabilité de la vitamine C.

Mots-clés : Vitamine C - Acide ascorbique - Stabilité - Catalyseurs - Conservation.

Ascorbic acid (vitamin C) is widely used in vitamin formulations and in other pharmaceutical preparations. It is a popular over-the-counter vitamin supplement throughout the world.

Chemically, ascorbic acid is an unsaturated lactone. It oxidizes much more rapidly in solution than in the solid state. Because of this, there has been a continued interest in its stability.

Essential to any kinetic study is a method for the quantitative determination of the reactants or products, alone or in the presence of interfering compounds.

An early method for the determination of l-ascorbic acid was based on a biological assay using guinea pigs [1]. One of the early standards for l-ascorbic acid was the « unit of vitamin C », one unit of which equalled the vitamin C activity of 0.1 ml of lemon juice, *Citrus limonum* [2]. Later, the 0.1 ml of lemon juice was replaced by 0.05 mg of l-ascorbic acid [3].

Since then many other analytical methods for the determination of ascorbic acid have been reported. Foremost amongst these are volumetric methods using dyes such as 2, 6-dichlorophenolindophenol; 2, 4-dinitrophenylhydrazine and iodine; chromatographic methods and spectrophotometric methods. An excellent review methods used for ascorbic acid determinations is given by HAJRATWALA [4].

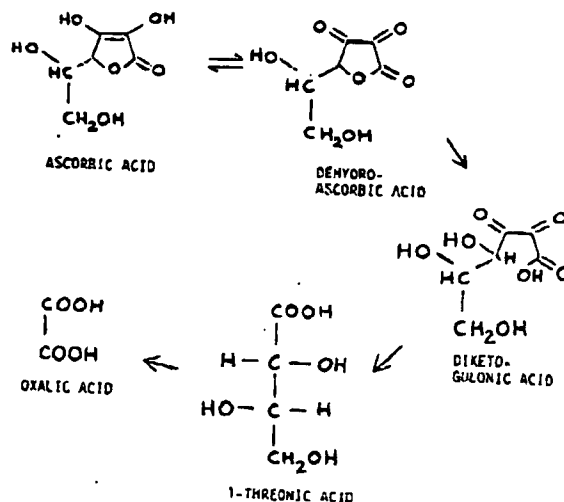
1. MAJOR ROUTES OF DEGRADATION AND DEGRADATION PRODUCTS

Since ascorbic acid oxidizes much more rapidly in solution than in the solid state, the stability of ascorbic acid solutions have received much more attention. Ascorbic acid degrades readily in aqueous solutions under aerobic as well as anaerobic conditions. The decomposition is more rapid under aerobic conditions than under anaerobic conditions.

1. Aerobic conditions

The degradation products for ascorbic acid are as follows [5].

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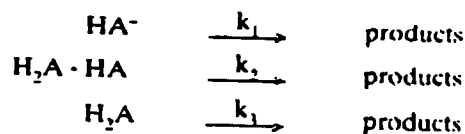


Ascorbic acid easily oxidizes, reversibly into dehydroascorbic acid. This oxidation step is dependent upon pH, oxygen content, and the presence of catalysts especially metal ions such as Cu^{+2} and Fe^{+3} .

The dehydroascorbic acid undergoes further hydrolysis to give the irreversible degradation product diketo-gulonic acid. This can undergo a series of oxidations to yield l-threonic acid and finally oxalic acid. All reactions are pH-dependent.

Kinetic scheme

The kinetic scheme postulated for the aerobic degradation of ascorbic acid is as follows [6].



Ascorbic acid is a diprotic weak acid represented by H_2A . HA^- is monodissociated ascorbic acid and $\text{H}_2\text{A} \cdot \text{HA}^-$ is a complex of undissociated ascorbic acid

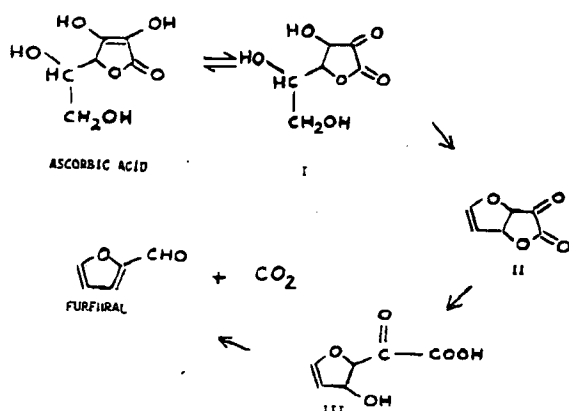
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and monohydrogen ascorbate. Such a complex successfully explained the observed pH-rate profile. However experimentally it was not possible to isolate such a complex. The rate equation for the above parallel reactions is

$$\text{rate} = k_1 [\text{HA}^-] + k_2 [\text{H}_2\text{A} \cdot \text{HA}^-] + k_3 [\text{H}_2\text{A}]$$

2. Anaerobic conditions

Under anaerobic conditions, the degradation products of ascorbic acid are different [7] :

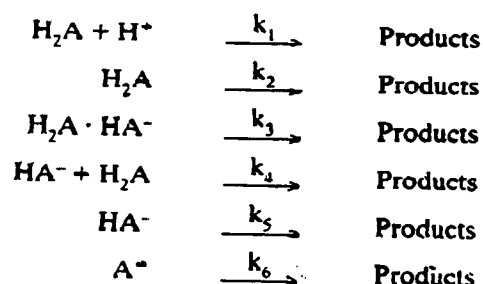


I, II & III are intermediates

Its main end products are furfural and carbon dioxide through a series of intermediates. These reactions are also pH dependent and are subject to catalysis by a variety of catalysts.

Kinetic scheme

The anaerobic degradation of ascorbic acid is explained by a set of parallel reactions.



The H_2A , HA^- and A^- are undissociated, monodissociated and didissociated species of ascorbic acid. In the above scheme the reactions 3 and 4 are kinetically equivalent, thus indistinguishable. They may be combined to yield one term; however the authors have chosen to use separate terms. The rate equation for the above set of parallel reactions is :

$$\text{rate} = k_1 [\text{H}_2\text{A}] [\text{H}^+] + k_2 [\text{H}_2\text{A}] + k_3 [\text{H}_2\text{A} \cdot \text{HA}^-] + k_4 [\text{HA}^-] [\text{H}_2\text{A}] + k_5 [\text{HA}^-] + k_6 [\text{A}^-]$$

For both aerobic as well as the anaerobic, degradation occurs via the pseudo first order, the rate being dependent upon the total ascorbic acid concentration.

3. Photochemical oxidation

The photochemical oxidation of ascorbic acid can occur under either aerobic or anaerobic conditions. X-ray and gamma irradiation are more effective than UV light irradiation, but all produce dehydroascorbic acid and hydrogen peroxide [8, 9]. The dehydroascorbic acid does not undergo photochemical decomposition.

4. Decarboxylation

Several researchers have shown that when ascorbic acid aqueous solutions are heated under anaerobic conditions, they undergo decarboxylation. It is known that metal ions catalyze decarboxylation of keto-acids. The mechanism of decarboxylation appears to be via a complex formation between metal ion and the di ion of the acid which undergoes rapid decarboxylation [10, 11].

II. pH INFLUENCE

1. pH-log rate profile

Figure 1 shows the pH-log rate profile for the ascorbic acid decomposition in aqueous solutions. The k is a pseudo-first order rate constant and was recalculated in seconds for ease of comparison. The studies were conducted at different temperatures and hence allowance must be made when comparing rate constants.

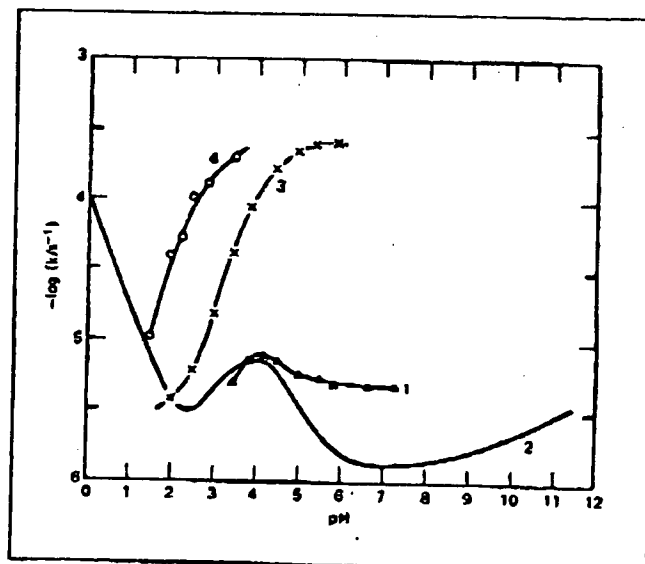


Figure 1 — pH-log rate profiles for ascorbic acid decomposition in aqueous solutions. Curve 1, aerobic, 67°C, $\mu = 0.4$ (Reference [6]); Curve 2, anaerobic, 96°C, $\mu = 0.5$ (Reference [7]); Curve 3, saturated with O_2 , 25°C, $\mu = 0.1$ (Reference [17]); Curve 4, saturated with O_2 and in the presence of $5 \times 10^{-4} \text{M Cu}^{+2}$, 25°C, $\mu = 0.1$ (Reference [17]). Reproduced from reference [5] with the permission of the copyright owner (John Wiley & Sons).

Curves 1 and 2 show aerobic and anaerobic decomposition respectively. Ascorbic acid decomposition shows minima around pH 2.5 to 3, followed by a maxima around pH 4.0 and another minima around pH 6.0 to 6.5.

Curve 3 shows the decomposition when a solution is saturated with oxygen. Curve 4 shows the decomposition in the presence of Cu^{++} when a solution is saturated with oxygen.

The pH-log rate profile shows a bell-shaped curve with a maxima near pH 4. The pK_a of ascorbic acid is reported to be 4.21 at 25.5°C and 3.98 at 67°C [6, 12, 13]. The bell shape profile is generated by the fact that the monoacid form of a diprotic acid is the reactive species. The pH-log rate profiles were explained by presupposing the existence of a complex formation between ascorbic acid and the monohydrogen ascorbate ion. It is well known that organic acids are able to form complexes with their anions [14, 15]. The possible complex formation between ascorbic acid and monohydrogen ascorbate was investigated using freezing point depression and boiling point elevation methods and solubility analysis [7]. These methods failed to prove the existence of such a complex; however, from a kinetic point of view, a reaction that presupposes the existence of an ascorbic acid-ascorbate complex can explain the observed pH-log rate profile.

2. Thermodynamic parameters

Table I shows the thermodynamic parameters for aerobic and anaerobic decomposition of ascorbic acid at different pH values. Different values of activation energies, E_a , at various pH values, indicate the possibility that a different reaction is a dominant one at different pH values [6, 16].

Table I — Thermodynamic parameters for ascorbic acid decomposition at different pH values.

a) at 60-85°C, ionic strength = 0.4 M, reference [6].
b) at 80-100°C, ionic strength = 0.5 M, reference [16].

pH	Aerobic ^a		Anaerobic ^b
	E_a (kcal/mole)	ΔS (cal/mole ⁻¹ deg ⁻¹)	E_a (kcal/mole)
0.38	—	—	19
3.52	12.2	-49.2	—
4.00	—	—	25
4.55	10.9	-52.1	—
5.45	10.1	-54.5	—
6.60	7.8	-61.7	—
7.50	—	—	24
11.38	—	—	23

The large negative values of entropy of activation, ΔS^\ddagger , indicates that the degree of disorders in the activated complex and in the reaction is great. Thus a simple atomic reaction can be ruled out for a mechanistic pathway of ascorbic acid oxidation.

III. METAL IONS AND OTHER CATALYSTS

1. Metal ions

Metal ions in general act as a catalyst for ascorbic acid decomposition. Figure 2 shows the effect of bivalent metal ions on the pseudo first order rate constant of the anaerobic degradation of ascorbic acid at different pH values at 96°C. Pb^{+2} was found to be the most powerful catalyst followed by Zn^{+2} , Co^{+2} , Fe^{+2} , Mn^{+2} , Ni^{+2} , Ca^{+2} and Mg^{+2} . A possible metal-ascorbate-oxygen complex is proposed as an intermediate species to explain the effect of metal ions on the aerobic oxidation of ascorbic acid [17]. Figure 3 shows such a complex. The rates of ferric and cupric ion catalyzed oxidations were found to be first order with respect to the concentrations of molecular oxygen. The catalytic activity of cupric ion was found to be more than that of ferric ion towards the ascorbate anion. However, for the oxidation of neutral species, the ferric ion was a better catalyst than the cupric ion. The catalytic activity of vanadyl ion was found to be less than that of ferric and cupric ions [18].

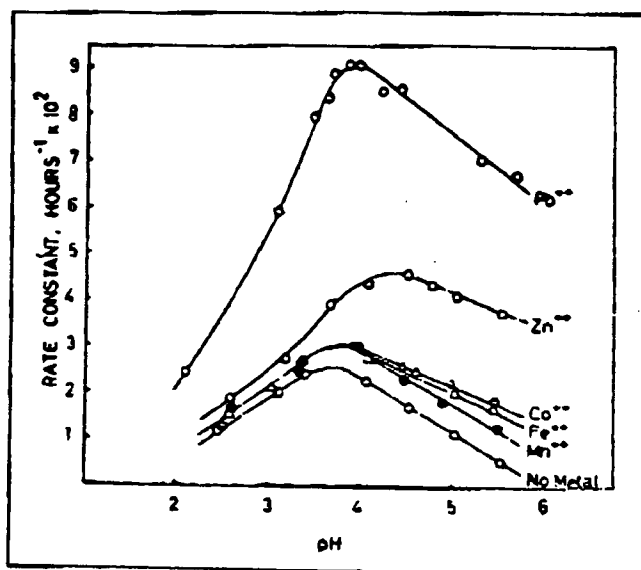


Figure 2 — Effect of bivalent metal ions on the pseudo first order rate constant of the anaerobic degradation of ascorbic acid, 96°C & $\mu = 0.05$. Reproduced from reference [10] with the permission of the copyright owner (J. Pharm. Sci.).

2. Other catalysts

Ascorbic acid is subject to general acid and general base catalysis under aerobic conditions. Figure 4 shows the effect of acetate concentration on the rate of oxidation of ascorbic acid at 67°C at various pHs.

Based upon the pK_a s of buffer acids, pK_a of ascorbic acid and the slopes of the line in figure 4, it can be determined which species are catalytic to ascorbic acid. Both undissociated and monodissociated acetic acid species are catalytic to both undissociated and monodissociated ascorbic acid species. The extent of catalysis depends upon pH. Trace quantities of metals catalyze the oxidation of ascorbic acid. Since buffers usually contain trace quantities of metal, the results shown in figure 4 could

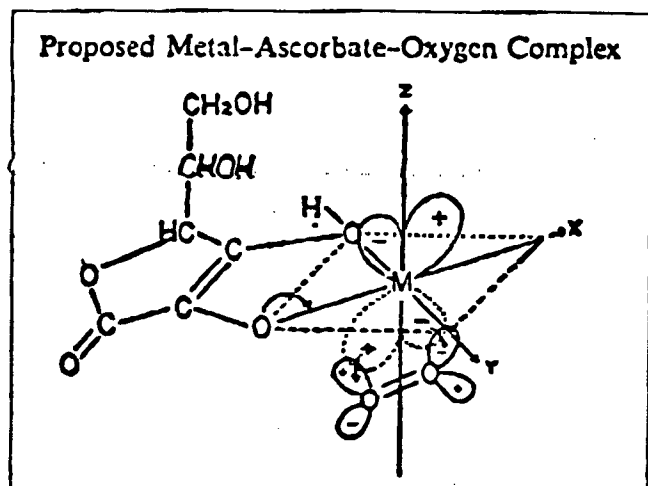


Figure 3 — Metal ascorbate-oxygen complex as proposed by TAQUIKHAN and MARTELL. Reproduced from reference [17] with the permission of the copyright owner (J. Am. Chem. Soc.).

be due to metal contamination. Oxidation was studied at the highest buffer concentration in the presence of disodium ethylenediaminetetraacetate. The results show the catalytic effect due to trace metal ions to be negligible.

The phosphate buffer species were also found to catalyze ascorbic acid decomposition under aerobic oxidation. Borate, oxalate, acetate, and phosphate buffer species were also catalytic under anaerobic conditions [7].

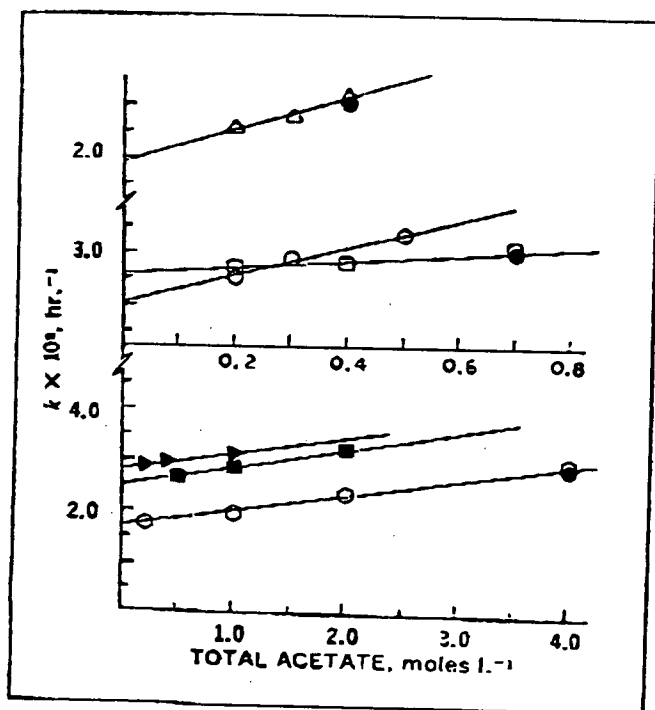


Figure 4 — Effect of acetate concentration on the rate and oxidation of ascorbic acid at 67°C at various pH's and ionic strength 0.4. Key: ○, pH 3.52; ●, pH 3.88; ▲, pH 4.25; □, pH 4.55; △, pH 5.05; and ●, with 0.1% disodium ethylenediaminetetraacetate. Reproduced from reference [6] with the permission of the copyright owner (J. Pharm. Sci.).

IV. EFFORTS TO INCREASE STABILITY

Many patents covering the stability aspect of *L*-ascorbic acid have been issued and likewise many papers covering ascorbic acid stability have been published. The following documentation is not an exhaustive list. Generally the following approaches have been used to prepare a stable ascorbic acid product.

1. Exclusion of air and oxygen

Ascorbic acid solution is kept under nitrogen or an inert gas atmosphere, in preferably small dosage units, usually for parenteral use [19]. Anaerobic degradation occurs much slower than aerobic degradation.

2. pH adjustment

The pH is adjusted at around 6 to 6.5, since this represents one of the minima in the pH-log rate profile [19]. The minima in the acidic range is generally not used since it causes tissue irritation.

3. Avoiding metal ion contamination

Several heavy metals are known to catalyze ascorbic acid decomposition. These are avoided using ultrapure water and reagents. Chelating agents such as ethylenediaminetetraacetic acid have been found to exert a stabilizing effect on ascorbic acid solutions [20, 21].

4. Light and temperature

Numerous studies [6, 7, 22] have shown increased ascorbic acid stability in the absence of light and at reduced temperatures. Since photochemical decomposition can occur under either aerobic or anaerobic conditions, absence of light is important.

5. Use of additives

Additives are used in ascorbic acid formulations for a number of reasons. Since numerous chemicals act as catalysts for ascorbic acid degradation, the selection of additives, matrices and solvents is important.

5.1. Solvents and matrices

Ascorbic acid was found to have a marked stability in high concentrations of a variety of solvents such as polyethylene glycol, propylene glycol, glycerin and sorbo. The optimum ratio of solvents varies amongst studies [20, 22, 23]. The increased stability is generally attributed to decreased dissolved oxygen content at higher concentrations of these solvents.

Ascorbic acid also undergoes autooxidation in the solid state, although the rate is much less than that in an aqueous solution. Degradation occurs via pseudo first order. When oxidized, ascorbic acid changes colour in the solid state from white to brown. Figure 5 shows that the reflectance measurements for ascorbic acid tablets can be used to determine first order rate constants in place of chemical assay for determining ascorbic acid content [24].

With solid formulations, diluent moisture content in excess of 1% is considered detrimental [25]. Ascorbic

acid formulations with either mannitol, sucrose or lactose as diluent did not show any changes in colour. The colour change was not universally indicative of a loss of vitamin potency. Tablets granulated with water or dilute alcohol discolored rapidly, however the assays of these tablets indicated no significant difference in stability.

A study of the effects of flavouring agents on the stability of ascorbic acid solutions showed the following order [26] : vanilla > raspberry > chocolate > cherry > pineapple > banana.

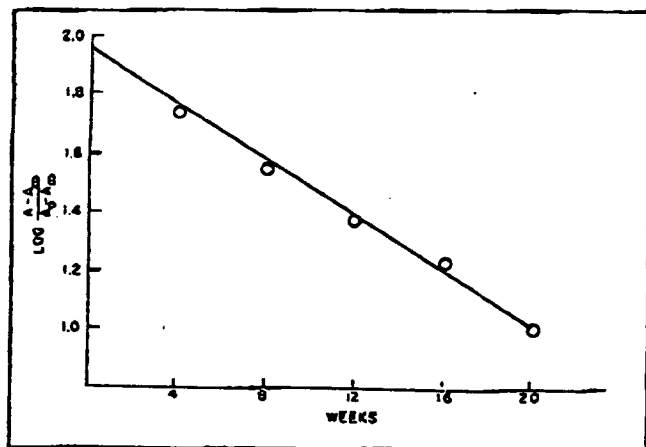


Figure 5 — Vitamin C degradation as followed by reflectance measurements. Reproduced from reference [24] with the permission of the copyright owner (Academic Press).

5.2. Antioxidants and chelating agents

Numerous compounds have been tested for their efficacy in stabilizing ascorbic acid solutions. An extensive review is given by HAJRATWALA [27]. Ethylenediaminetetraacetic acid is one of the most popular, as well as efficient chelating agents. Most proteins and amino acids act as noncompetitive inhibitors, either through the formation of stable ascorbic acid-protein or cupric-protein complexes.

5.3. Surfactants

There have been many reports concerning the increase in the stability of drugs in aqueous solutions in the presence of surfactants. When surfactants or surface active agents are dissolved in an aqueous solution, they orient themselves at the surface of the liquid. Any excess surfactant which cannot be adsorbed at the surface of the liquid will remain in the bulk of the solution. Here it forms micelles or colloidal aggregates. The increased stability can often be attributed to these micelles.

Figure 6 shows the oxidation of saturated solutions of copper catalyzed ascorbic acid in the presence of polysorbate 20. The oxidation rate of ascorbic acid declined rapidly between 40 and 75 % surfactant. This decrease in the rate at high concentrations of surfactant was attributed to a decrease in the diffusion of oxygen to the site of oxygen due to the high viscosity of the solution [28].

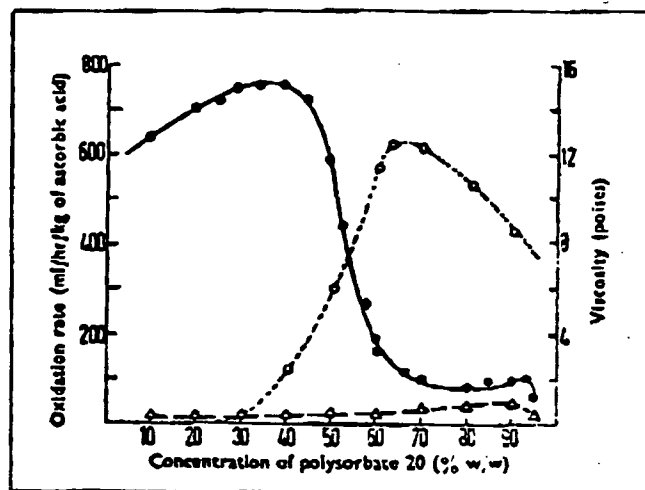


Figure 6 — The oxidation of saturated solutions of ascorbic acid in polysorbate 20, at 25°C & pH 3.4. Key : —, catalyst $1 \times 10^{-4}M$ $CuSO_4 \cdot 5H_2O$; ... uncatalyzed; ---, viscosity. Reproduced from reference [28] with the permission of the copyright owner (J. Pharm. Pharmacol).

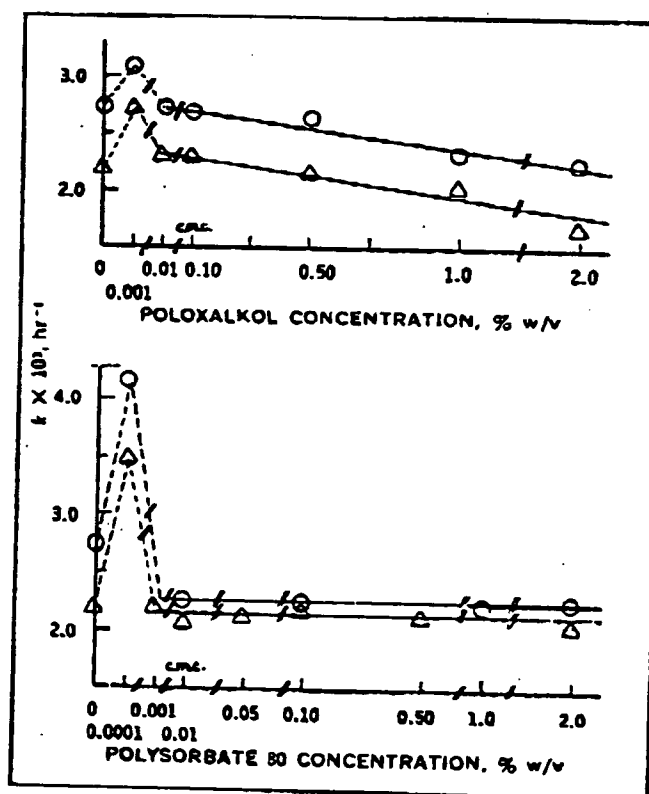


Figure 7 — Effect of various concentrations of surfactants on the rate of oxidation of ascorbic acid at 67°C and $\mu = 0.4$. Key : Δ , pH 4.55 (acetate buffer); \circ , pH 6.60 (phosphate buffer). Reproduced from reference [30] with the permission of the copyright owner (J. Pharm. Sci.).

In the presence of polysorbate 80, a rapid decline in the rate of oxidation of ascorbic acid was reported in solutions containing 3 % ascorbic acid and varying concentrations of polysorbate 80. The rate declined in solutions containing up to 10 % surfactant and remained constant at surfactant concentrations between 10 and 30 % [29]. The decrease in the oxidation rate of

ascorbic acid was attributed to an increase in both micelle concentration and micelle aggregation in solutions containing up to 10 % polysorbate 80. The increase in viscosity produced at high surfactant concentration had no significant effect on the rate of oxidation of ascorbic acid.

Figure 7 shows the effect of two nonionic surfactants at low concentrations on the oxidation rate of ascorbic acid [30]. The rate increased sharply at one hundredth of critical micelle concentration (CMC) of the surfactant. This is attributed to the adsorption of ascorbic acid molecules on the surface of the surfactant molecules, making them more susceptible to oxidative attack through surface catalysis. The increase could also be due to the formation of our association complex between ascorbic acid and surfactant molecules. The difference in the behaviour of two surfactants may be due to differences in aggregation number and size of micelles, or in the number of available sites in the surfactant molecules at which ascorbic acid may bind. ■

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MANU RIPT

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EXHIBIT E

PHARMACEUTICA ACTA HELVETIAE

Wissenschaftliche Zeitschrift des schweizerischen Apothekervereins

Périodique scientifique de la Société Suisse de Pharmacie

Redaktion: Prof. Dr. J. Büchi
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National Research Centre, Dokki, Cairo, UAR
Laboratory of Pharmaceutical Sciences

Studies on the stability of injectable L-ascorbic acid solutions

I. Effect of pH, solvent, light and container

By *M. A. Kassem, A. A. Kassem and H. O. Ammar*

(Received January 24, 1969)

The literature dealing with the stability of L-ascorbic acid in solution is rather intensive and covers different aspects, some of which constitute those considered in this communication. However, unique conclusions of the different reports are rather scarce. This is most prominent for the pH-effect, where surprisingly contradictory results were reported. Optima of stability were reported to exist at the pH-values of 3¹; 4,6²; 6³; 6,3^{4, 5} and 7^{6, 7}.

Concerning the solvent effect, few investigations have dealt with merits of the use of water-miscible organic solvents, namely, polyhydric alcohols and glycols in parenteral L-ascorbic acid solutions. Propylene glycol was reported to possess a

¹ *Finholt P., Alsos J. and Higuchi T.*, J. pharm. Sci. [Washington] 54, 181 (1965).

² *Baczyk S., Lempka A. and Baranowska K.*, Pr. Zakresu Towarozn. Chem., Wyssza Szk. Ekon. W. Poznaniu Zesz. Nauk. Ser. I, No. 26 (1966).

³ *Egawa S.*, Yakuzaijaku 21, 177 (1961).

⁴ *Bartilucci A. and Foss N.E.*, J. amer. pharm. Ass. (sci. Ed.) 43, 159 (1954).

⁵ *Nicolas G.*, Rev. farm. [Buenos Aires] 93, 124 (1951).

⁶ *Conrado S.*, An. Fac. Farm. Univ. Recife 4, 161 (1961).

⁷ *Shnaidman L.O.*, Vesesoyuz. Nauch.-Issledovatel. Vitamin Inst. Vitaminizatsiya Pishchevykh Productov 1955, 43.

stabilizing effect⁸⁻¹². On the other hand, few investigations reported a stabilizing effect for ethylene-, butylene- and polyethylene glycols as well as for glycerol and sorbitol^{8, 12-17}.

As far as the light effect is concerned, *Nogueira*¹⁸ and *Géro*¹⁹ reported that light possesses no deleterious effect towards L-ascorbic acid solutions. On the other hand, *Martini*^{20, 21} and *Guinand*²² showed that light accelerates the decomposition.

Considering the above mentioned citations, it was found interesting to investigate how the effects of pH, glycols, light and container would reflect themselves on the degradation rate of L-ascorbic acid in injectable solutions. Furthermore, the comparative evaluation of certain inorganic and organic bases as regards their effect in this respect was deemed necessary, in view of the fact that the use of such agents for adjusting the pH of these solutions is appraised²³.

Experimental

1. Effect of pH, inorganic and organic bases

10 per cent solutions of L-ascorbic acid* in deionized water** were prepared; the pH of the solutions was adjusted with sodium bicarbonate to values covering the range of 2.6-8. The solutions were then filtered and filled in brown glass ampoules, 5 ml each. For each solution, part of the ampoules was sealed directly, and the other was sealed after displacing the dissolved air, as well as the air space over the solution by purified nitrogen gas†. The ampoules were sterilized by autoclaving at 116° for 30 min and then kept in an incubator at 50°. The L-ascorbic acid content of the different

* Supplied by HOFFMANN-LA ROCHE.

** Specific resistance = > 4 Megohm · cm. In virtue of the constancy of the amounts of trace metal ions - which were found to be extremely small - present in deionized water, this water was preferred to bidistilled water in the present investigation²⁴.

† Purified by passage through 4 traps of alkaline pyrogallol solution and a trap of sulphuric acid for dehydration.

⁸ *Hüttenrauch R.*, Pharm. Zhalle 99, 739 (1960).

⁹ *Joslyn M.A. and Miller J.*, Food Res. 14, 325 (1949).

¹⁰ *Rao J.*, Pharm. J. 158, 143 (1947).

¹¹ *Zwolinska Z.*, Farm. polska 18, 548 (1962).

¹² *Kato Y.*, Yakuzaigaku 25, 131 (1965).

¹³ *Stan M. and Zehlinger R.*, An. Univ. Bucuresti, Ser. Stiint. nat. 1957, No. 16, 79.

¹⁴ *Agrawal D.K., Sen R., Upreti M.C., Sen N. and Rao V.K.M.*, Indian J. Technol. 1, 90 (1963).

¹⁵ *Upreti M.C., Sen R. and Rao V.K.M.*, J. sci. ind. Res. [New Dehli] 19 C, 249 (1960).

¹⁶ *Upreti M.C., Sen R., Agrawal D.K. and Rao V.K.M.*, Indian J. Technol. 1, 391 (1963).

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¹⁸ *Nogueira A.L., Faria M.J. and Ruivo M.L.S.*, Rev. port. Farm. 13, 16 (1963).

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²³ *Lautenschläger C.L. and Linder F.*, DRP 699327 (1940); US Pat. 2249903 (1940).

²⁴ *Ammar H.O.*, A study of certain technological aspects concerning the stability of injectable L-ascorbic acid solutions, M. Pharm. thesis University of Cairo, 1968.

solutions was estimated before and after autoclaving as well as every week later using the N-bromosuccinimide method²⁵.

Concerning the effect of inorganic and organic bases, the stability of 10 per cent L-ascorbic acid solutions was investigated in presence of each of triethanolamine (pH=6; 6,5 and 7), calcium carbonate and sodium carbonate (pH=4,8).

2. Effect of glycols

Here, 1,2-propylene glycol and polyethylene glycol 400 were considered. The stability of 10 per cent L-ascorbic acid solutions (pH=6,3) in glycol-water systems (5-50 % glycol) was investigated at 50°.

3. Effect of light and container

A 10 per cent aqueous L-ascorbic acid solution (pH=2,6) was prepared and filled in colourless and in dark brown glass ampoules, 5 ml each. The ampoules were sealed, sterilized and divided into two groups, the first group was kept in colourless glass jars, while the second was kept in carton boxes. The two groups were kept in direct sunlight at room temperature. The L-ascorbic acid content of the different batches was determined periodically.

Results and discussion

1. Effect of pH

On autoclaving, it is obvious that a pH-dependent loss of L-ascorbic acid takes place (table 1). The highest loss in potency takes place at pH = 4; in the stronger and weaker acid sides, the loss in potency decreases once again until it becomes minimum at pH = 6,5. At pH-values higher than the latter, the loss increases once again in proportion to the pH-value. In solutions kept under nitrogen, the decomposition of L-ascorbic acid is less than that observed in the respective solutions kept under air, the effect of nitrogen being most remarkable at the low pH-values.

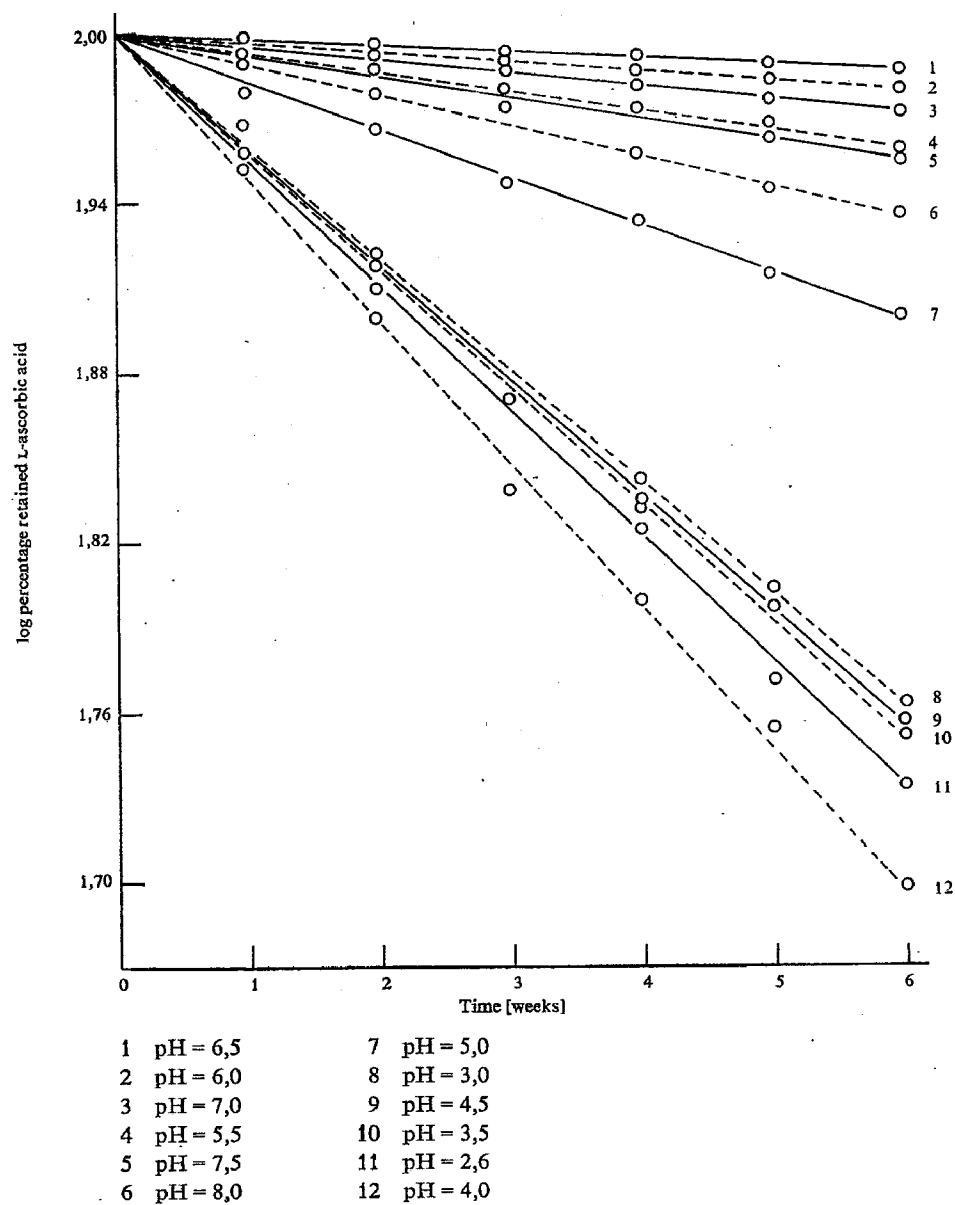
The degradation of L-ascorbic acid in the investigated solutions, is found to take place according to first-order reaction kinetics (fig. 1 and 2); neither the pH of the solutions nor the nature of the atmosphere under which they are kept is of influence on the order of the reaction. Accordingly, the finding of Peterson²⁶, that the order of the reaction is pH-dependent, could not be confirmed.

The reaction rate constant (k) is found to be highly pH-dependent (table 2 and fig. 3). The highest values of k are observed in the pH range 2,6 to 4,5. However, a minimum occurs at pH = 3, which thus represents a small maximum of stability in the above mentioned range. The reaction rate attains its highest value at pH = 4. Beyond the latter value, the reaction rate constant of L-ascorbic acid decreases greatly and progressively until it reaches the minimum value at pH = 6,5. At pH-values higher than the latter, the stability decreases once again as shown by the sharp increase of the k-value. If the k-values at the two stability maxima (pH = 3 and pH = 6,5) are compared, it would be obvious that the rate of degradation of L-ascorbic acid at pH = 6,5 is only about 5,5 per cent of that at pH = 3.

²⁵ Barakat M. Z., Abd-El-Wahab M. F. and El-Sadr M. M., Anal. Chem. 27, 536 (1955).

²⁶ Peterson R. W. and Walton J. H., J. amer. chem. Soc. 65, 1212 (1943).

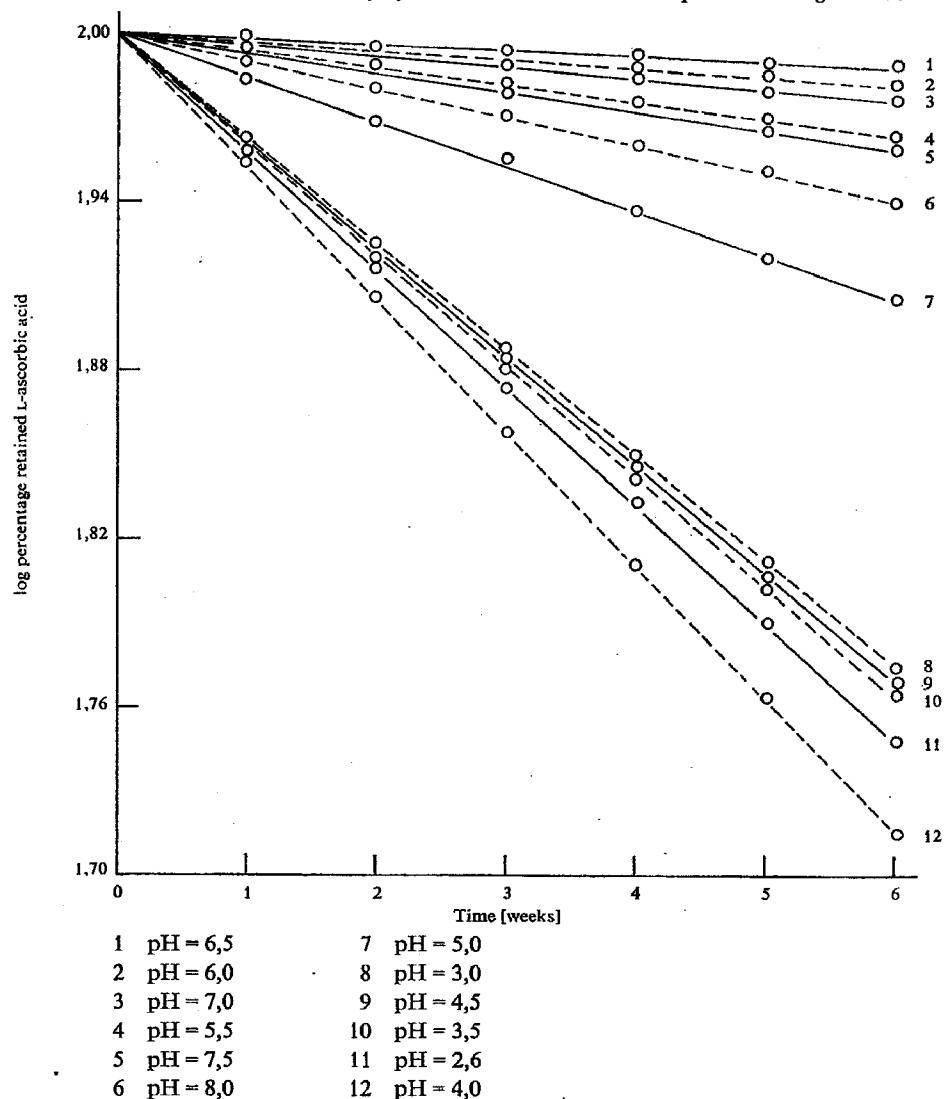
Figure 1

Effect of pH on the stability of L-ascorbic acid solutions kept at 50°

2. Effect of inorganic and organic bases

It can be noticed from tables 2 and 3 and fig. 4 that the stability of solutions adjusted with sodium bicarbonate is higher than that of solutions adjusted with

Figure 2

Effect of pH on the stability of L-ascorbic acid solutions kept under nitrogen at 50°

triethanolamine to the respective pH-values. In the investigated pH range, the dependency of the stability of L-ascorbic acid on pH is the same in both cases.

It is also obvious that the stability of L-ascorbic acid solutions treated with calcium carbonate is slightly better than that of solutions treated with sodium carbonate (table 3 and fig. 5). This finding may be explained on the basis of the low dissociation constant of calcium ascorbate²⁷.

²⁷ Veselinovic D.S. and Susic M.V., Glasn. hem. Društ. [Beograd] 30, 63, 79 (1965).

Table 1

Effect of pH on the stability of L-ascorbic acid solutions during autoclaving

pH-Value	% Loss in L-Ascorbic Acid	
	Solutions kept under air	Solutions kept under nitrogen
2,6	2,00	1,40
3,0	3,43	2,02
3,5	3,88	2,99
4,0	8,75	8,44
4,5	7,40	5,42
5,0	3,77	3,08
5,5	3,00	2,40
6,0	2,30	1,60
6,5	1,30	0,90
7,0	1,50	1,00
7,5	2,28	1,98
8,0	2,62	1,99

Table 2

Effect of pH on k of L-ascorbic acid in solutions, kept at 50°

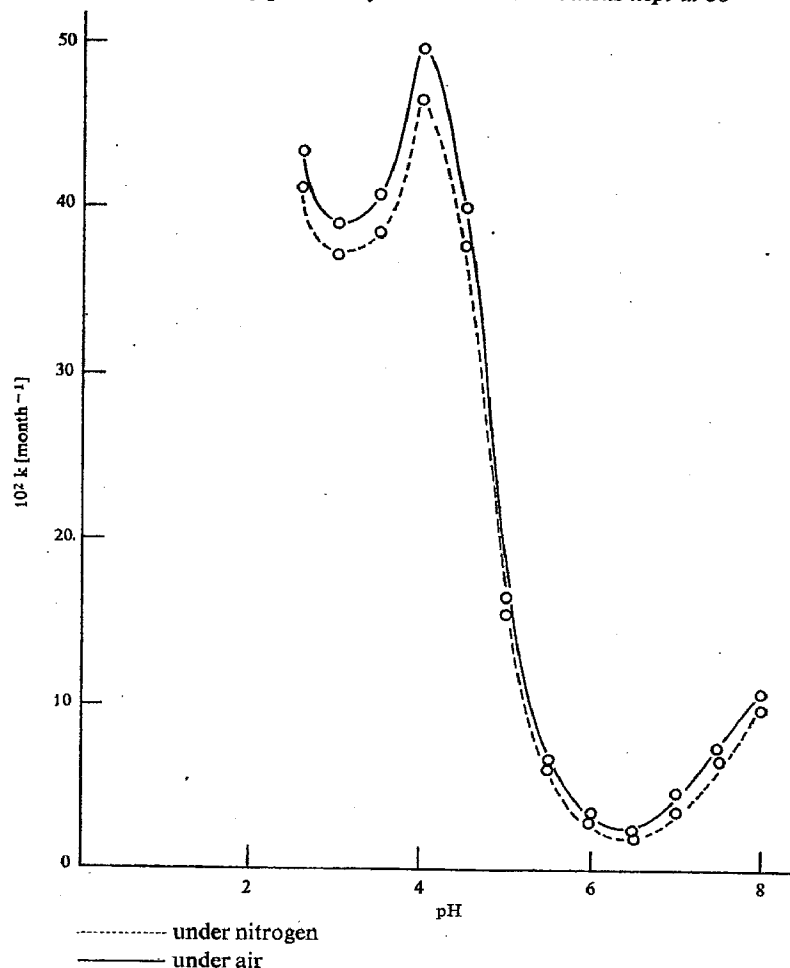
pH-value	k [month ⁻¹]	
	Solutions kept under air	Solutions kept under nitrogen
2,6	0,4361	0,4126
3,0	0,3908	0,3703
3,5	0,4076	0,3861
4,0	0,4955	0,4662
4,5	0,4001	0,3780
5,0	0,1653	0,1564
5,5	0,0684	0,0614
6,0	0,0355	0,0299
6,5	0,0229	0,0196
7,0	0,0472	0,0340
7,5	0,0749	0,0670
8,0	0,1073	0,0982

Table 3

Effect of inorganic and organic bases on k of L-ascorbic acid in solutions, kept at 50°

Base	pH of solutions	k [month ⁻¹]
Triethanolamine	6,0	0,0561
Triethanolamine	6,5	0,0416
Triethanolamine	7,0	0,0656
Calcium carbonate	4,8	0,1989
Sodium carbonate	4,8	0,2066

Figure 3
Effect of pH on k of L-ascorbic acid solutions kept at 50°



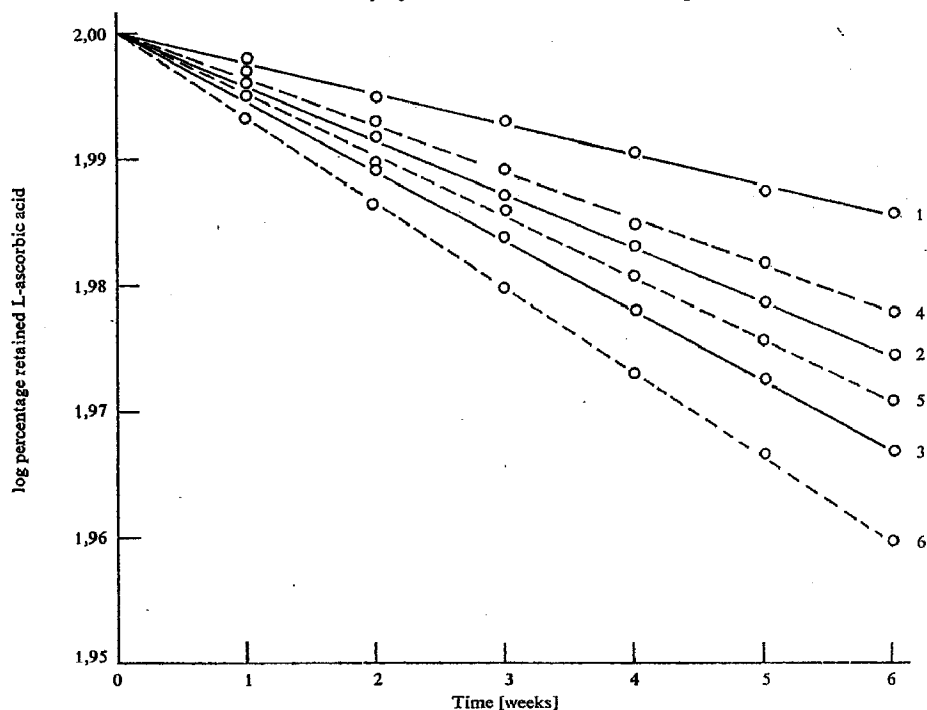
3. Effect of glycols

It is obvious from table 4 that both 1,2-propylene glycol and polyethylene glycol 400 protect L-ascorbic acid during autoclaving. The higher the glycol concentration, the higher is the stability of L-ascorbic acid. On the other hand, it is also seen that 1,2-propylene glycol offers a fairly better protection to L-ascorbic acid during autoclaving than does polyethylene glycol 400.

Fig. 6 and 7 show that the degradation of L-ascorbic acid in these solutions takes place according to first-order reaction kinetics. The presentation of the k -values for L-ascorbic acid, as a function of percentage glycol concentration, shows that the reaction rate decreases linearly with increasing glycol concentration from 5 to 50 per cent (fig. 8). It is also seen that by increasing the glycol concentration from 0 to 5 per

Figure 4

Effect of triethanolamine and sodium hydrogencarbonate on the stability of L-ascorbic acid solutions kept at 50°



- | | |
|--------------------------------------|-----------------------------|
| 1 Sodium hydrogencarbonate; pH = 6,5 | 4 Triethanolamine; pH = 6,5 |
| 2 Sodium hydrogencarbonate; pH = 6,0 | 5 Triethanolamine; pH = 6,0 |
| 3 Sodium hydrogencarbonate; pH = 7,0 | 6 Triethanolamine; pH = 7,0 |

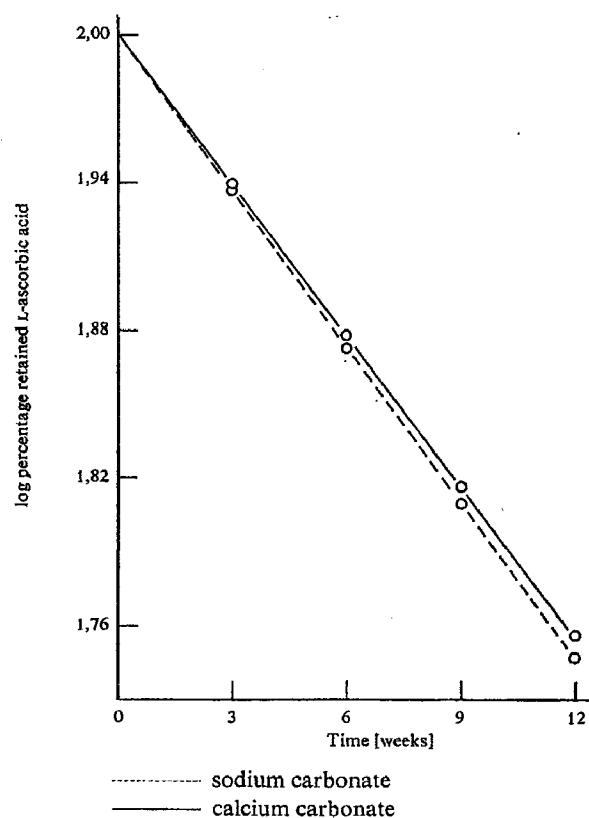
Table 4

Effect of glycols on the stability of L-ascorbic acid solutions during autoclaving

Glycol [per cent, v/v]	% Loss in L-ascorbic acid in presence of	
	1,2-propylene glycol	polyethylene glycol 400
0	2,68	2,68
5	1,19	1,99
10	1,00	1,70
25	0,81	1,38
50	0,72	1,17

cent, the decrease in the reaction rate constant is most significant; by the further increase of the glycol concentration, the effect becomes less significant. On the other hand, it is also evident that the stabilizing effect of 1,2-propylene glycol is higher than

Figure 5
*Effect of calcium carbonate and sodium carbonate on the stability of
L-ascorbic acid solutions kept at 50°*



that of polyethylene glycol 400. The slightly increased negative slope of the curve in the case of 1,2-propylene glycol indicates that the concentration effect of this glycol is more pronounced than that of polyethylene glycol 400. The curve also illustrates that 1,2-propylene glycol, in a concentration of 10 per cent, exhibits a stabilizing effect which is very near to that of polyethylene glycol 400 in a concentration of 25 per cent.

4. Effect of light and container

Fig. 9 shows that the stability of L-ascorbic acid solution in both colourless and brown glass ampoules kept in carton boxes is better than that of the same ampoules kept in glass jars. This shows that light has a deleterious effect towards the stability of L-ascorbic acid solutions. It is also observed that the stability of L-ascorbic acid solution kept in the colourless glass ampoules is better than that in the brown ones. This is, most probably, due to the higher content of heavy metal ions in the brown

Figure 6

Effect of 1,2-propylene glycol on the stability of L-ascorbic acid solutions kept at 50°

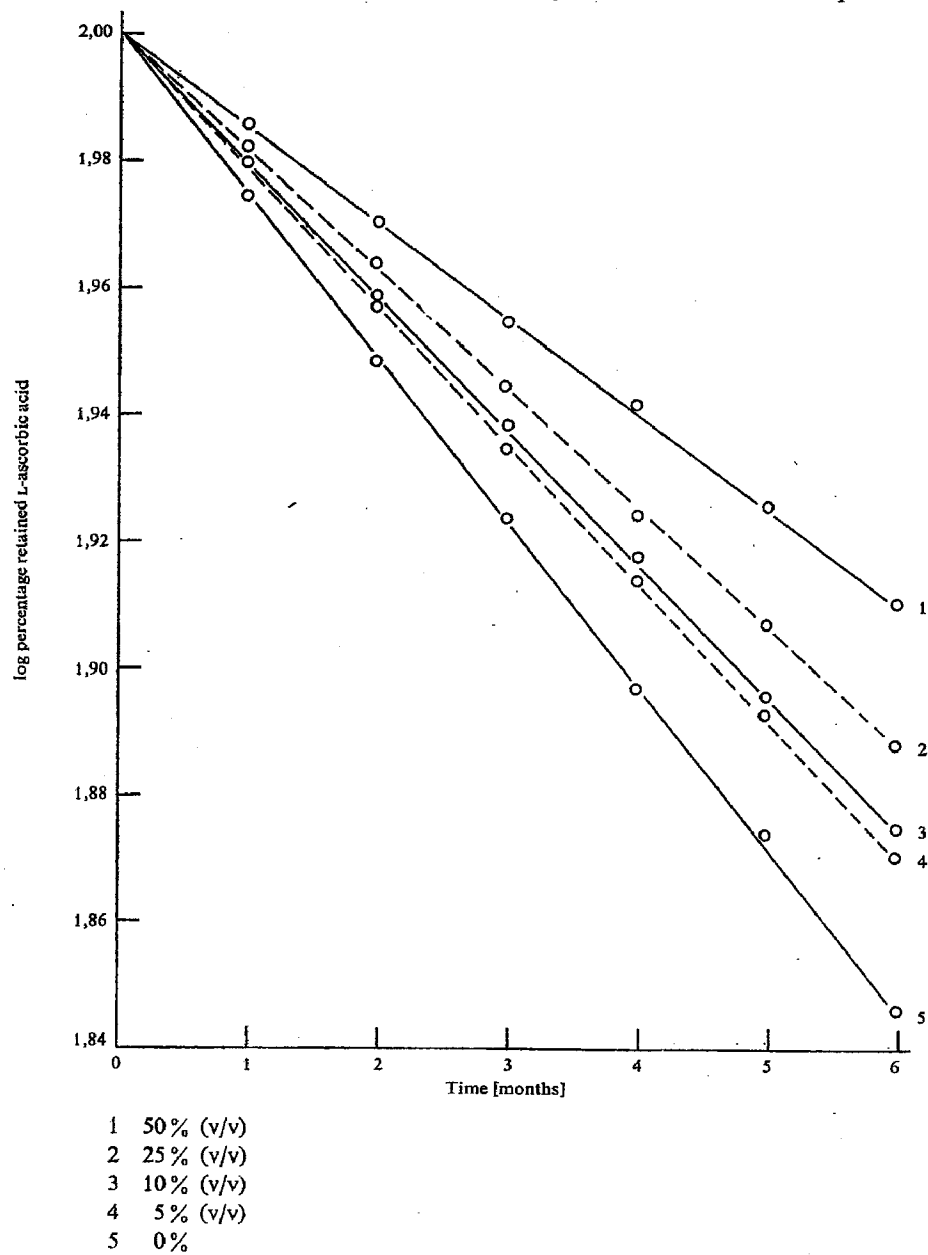


Figure 7
*Effect of polyethylene glycol 400 on the stability
of L-ascorbic acid solutions kept at 50°*

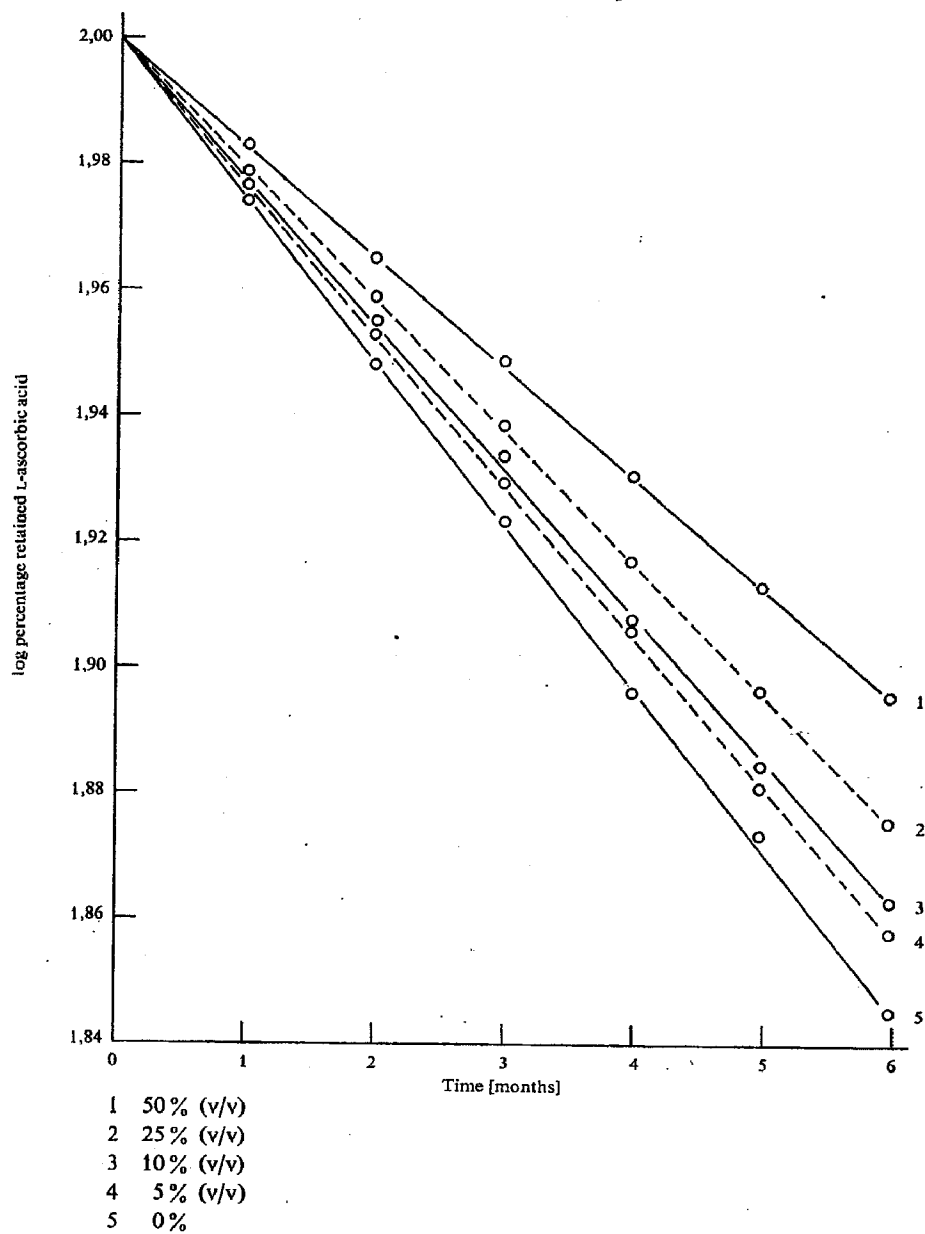
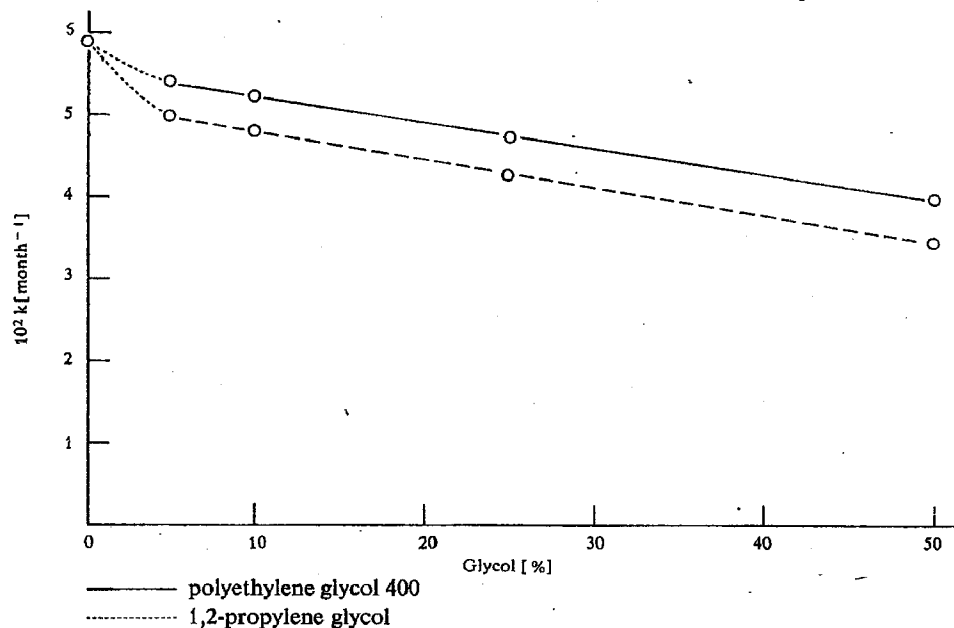


Figure 8

Effect of glycol concentration on k of L-ascorbic acid in solutions kept at 50°

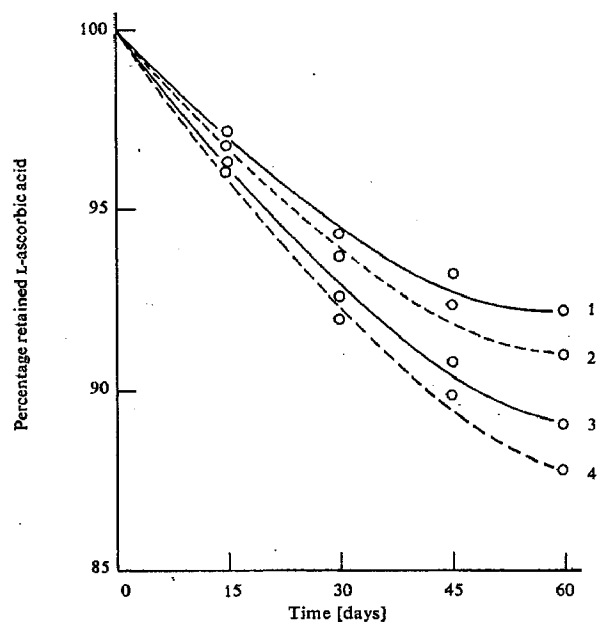
glass ampoules²⁴. However, it is observed that the light effect is more pronounced than the influence of the type of ampoule. On the other hand, the use of brown glass ampoules instead of the colourless ones, with the purpose of protecting L-ascorbic acid solutions against light, is found to offer no advantage.

Summary

1. The stability of L-ascorbic acid solutions is highly dependent on the pH. In the pH range of 2,6 to 4,5 the stability is very low with a minimum at pH = 4. Beyond pH = 4, the stability is increased reaching its absolute maximum at pH = 6 to pH = 6,5; beyond which the stability decreases once again.
2. The degradation of L-ascorbic acid in solution proceeds as a first-order reaction independent on the pH or the nature of atmosphere under which the solution is kept.
3. For adjusting the pH of L-ascorbic acid solutions, the use of sodium bicarbonate is superior to the use of triethanolamine. On the other hand, in presence of calcium ions, the stability is only slightly better than that in presence of sodium ions.
4. 1,2-propylene glycol and polyethylene glycol 400 – in proportion to their concentration – exhibit a stabilizing effect towards L-ascorbic acid solutions, the former being more efficient than the latter. The reaction rate constant of L-ascorbic acid decreases linearly with increasing glycol concentration in the range of 5 to 50 per cent.

Figure 9

Effect of light and container on the stability of L-ascorbic acid solutions kept at room temperature



- 1 Colourless ampoules in carton boxes
- 2 Brown ampoules in carton boxes
- 3 Colourless ampoules in glass jars
- 4 Brown ampoules in glass jars

5. Light has a deleterious effect on the stability of L-ascorbic acid solutions. The use of brown glass ampoules instead of colourless ones for keeping the solutions does not offer any advantage.

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